MONOGRAPHS

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The Lymphomas: Current Concepts in Pathogenesis and Management

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The Lymphomas: Current Concepts in Pathogenesis and Management

Conference

at the

Georgetown University Medical Center

Washington, D.C.

September 21-23, 1989

Sponsors:

Michele Susan Kogod Memorial Foundation
National Cancer Institute
Lombardi Cancer Research Center
Georgetown University Medical Center
Team Care

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Introduction

Marc E. Lippman[1] *and* **Douglas Yee**[2]

Hodgkin's disease and the non-Hodgkin's lymphomas have served as models for solid tumor oncology. Much of the way we approach solid tumors is based on principles learned from the management of the lymphomas. Researchers have made scientific and therapeutic advances by studying the lymphomas, and their continued progress in these diseases will apply broadly to the diagnosis and treatment of cancer.

This issue presents the proceedings of a conference, The Lymphomas: Current Concepts in Pathogenesis and Management, held at the Georgetown University Medical Center on September 21–23, 1989, Washington, D.C., and sponsored by the Lombardi Cancer Research Center, Michele Susan Kogod Memorial Foundation, National Cancer Institute, and Team Care. The aim of conference leaders was to have participants assess the current status of the diagnosis and treatment of the lymphomas and examine future directions in lymphoma management.

The molecular pathology and cytogenetics of the lymphomas represent research areas that are being investigated so that insight into the events that characterize malignant transformation will be provided to researchers and practitioners. Dr. Cossman discusses molecular events that occur in lymphoma and how their detection may be useful in the management of the patient. Drs. Levine and Bloomfield examine the chromosomal translocations that are found in the lymphomas and how they may provide prognostic information. These types of research are, in effect, a way to diagnose and stage the patient. The more traditional anatomical staging procedures, and their pitfalls, are examined by Drs. Raubitschek, Goffman, and Glatstein.

Therapeutic decisions are based on the pathologic and anatomic examination of the tumor. Drs. DeVita and Longo and Ms. Hubbard review the management of the patient with Hodgkin's disease with particular emphasis on chemotherapy, and Drs. Urba, Duffey, and Longo discuss the evaluation and treatment of the patient with the aggressive lymphoma. These two areas represent the most successful application of curative combination chemotherapy in the treatment of solid tumors. However, with the increasing success of treatment of these diseases has come the increasing awareness that long-term complications of therapy are ever present. The long-term survival of patients treated for Hodgkin's disease has allowed us to identify the toxic effects of chemotherapy and radiation therapy. Dr. Young discusses these problems and potential ways to avoid them. The AIDS epidemic has defined a subgroup of lymphoma patients who require different management strategies; Dr. Freter examines current treatment of the patient with AIDS-associated lymphoma.

Because the lymphomas are responsive to a variety of agents, novel therapeutic strategies have been tested in the treatment of these diseases. Drs. Levy and Miller discuss their experience in the production of anti-idiotype antibodies and their use in the treatment of indolent lymphomas. The current use and future possibilities for interleukin-2 as a therapeutic modality in lymphoma are presented in an article by Drs. Sondel and Malkovska. With considerable interest focused on the use of colony-stimulating factors in cancer treatment, Drs. Gabrilove and Jakubowski relate some of their results with using these agents to decrease the toxic effects of drugs on the bone marrow, while increasing the dose intensity of chemotherapy. Dr. Armitage and his co-workers describe their experience with bone marrow transplantation as salvage therapy for the lymphomas. Finally, Dr. Rubinow explores the ability of the central nervous system to influence immunologic and neuroendocrine events that may have an impact on immunologic function and potentially influence the response to cancer treatment.

These papers give us insight into the evolution of lymphoma treatment, the current status of diagnosis and therapy, and glimpses at future directions in therapy. Clearly, treatment of the lymphomas has been a success story for modern oncology. This conference highlighted the areas that need further investigation: an understanding of the genetic events that characterize malignant transformation, optimization of the chemotherapeutic regimens in regard to dose intensity, and use of biologically active agents, alone or in combination with chemotherapy, in treatment of these diseases. One challenge for oncologists in the 1990s will be to expand the progress already made in these diseases and to provide new insights that will be applicable to all types of solid tumors.

The conference organizers gratefully acknowledge the educational grants in support of this program from Adria Laboratories, Inc., the Michele Susan Kogod Memorial Foundation, and Team Care.

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Molecular Genetic Basis of the Diagnosis of Hematopoietic Neoplasms

Jeffrey Cossman[1]

ABSTRACT—New discoveries regarding the molecular genetic basis of lymphoma have begun to shape a model of lymphomagenesis that affords investigators an opportunity to measure the clinical effects of specific altered genes in a major form of human cancer.—J Natl Cancer Inst Monogr 10:3–6, 1990.

Malignant lymphomas and leukemias are a heterogeneous group of neoplasms derived from cells of hematopoietic origin that manifest a wide range of clinical behaviors. Indeed, clinical behavior and sensitivity to therapeutic intervention are consequences of innate biologic characteristics of the neoplastic cells and the molecular pathways associated with malignant transformation. In this regard, investigators have long sought cellular features, associated with the malignant phenotype, that might be predictive of prognosis. Morphologic hallmarks detectable at the light microscopic level have provided a basis for our detecting neoplasms and for distinguishing among classes associated with different clinical outcomes. However, because malignant neoplasms may closely resemble benign cellular proliferative processes, the diagnosis of lymphoma and leukemia remains a challenging area of histopathology. Furthermore, distinction of subtypes of hematopoietic neoplasms can be difficult because the morphologic overlap may be considerable. Therefore, we need independent biologic markers to aid us in the detection and classification of lymphoma and leukemia. Immunologic phenotyping methods have significantly aided routine morphologic diagnosis, but such approaches can be hampered by technical artifacts, inconsistencies of gene expression, and lack of clonal markers. This report is an outline of how nucleic acid probe hybridization assays circumvent such obstacles when applied to the diagnosis of hematopoietic neoplasms.

Although an individual neoplasm may be polymorphous and contain a variety of cell sizes, shapes, and features, it is now evident that nearly all malignant lymphomas and leukemias are monoclonal and are composed of nearly genetically identical progeny derived from a common clonal stem cell (*1*). For example, an abnormal chromosome, not carried in the germ line, can be found throughout a neoplasm but not in the normal cells of the same individual, e.g., the Philadelphia chromosome translocation t(9;21) of chronic myelogenous leukemia (*2*). Clonality, a consistent feature of most lymphomas and leukemias, is typical of solid tumors as well.

Associated with the clonality of neoplasms are abnormalities within the genome of the neoplastic cell. These may appear as gross morphologic changes in chromosomes, such as the translocation described above, or may be more subtle changes, such as DNA amplification, viral insertion, DNA deletion, or even point mutation. Many such genetic lesions have been shown to be mechanistically at the root cause of malignant transformation. Important from a diagnostic standpoint is the fact that genetic alterations may precede clonal expansion. Consequently, the daughter cells comprising the neoplasm each contains a genetic abnormality that can therefore serve as a tumor marker. Finally, classes of genetic abnormalities are nonrandomly associated with specific subtypes of lymphoma and leukemia (again, note the example of the Philadelphia chromosome associated with chronic myelogenous leukemia), as well as other prominent examples, such as the t(8;14) translocation of Burkitt's lymphoma and the t(14;18) of follicular lymphoma (*1*). Through the use of DNA cloning technology, the specific DNA sequences affected in the various neoplasms have been identified, and these regions of DNA can be cloned and used as probes to detect similar abnormalities in the neoplastic cells of other patients.

Lymphoid neoplasms carry an additional set of unique tumor markers beyond the genetic lesions described above. This category of markers is the rearranged antigen-receptor genes of B and T lymphocytes. Because most non-Hodgkin's lymphomas and lymphoid leukemias are neoplasms derived from either B or T cells, they have, in most instances, recapitulated the physiologic process of antigen-receptor gene rearrangement seen in early normal lymphoid cell development. The rearrangement of immunoglobulin genes in B cells and T-cell antigen-receptor genes in T cells affords the immune system with a recombinational process that greatly amplifies the number of possible sequences encoding the variable regions of the antigen-receptor genes. Through the process of rearrangement, point mutation, and assembly of two chains to form an antigen-receptor structure, the immune system can display an enormous array of antigen receptors available to meet foreign antigenic challenge (*3*). Antigen receptors are expressed on the cell surfaces of B cells as immunoglobulins or on T cells as T-cell receptors. Importantly, rearrangements occur early in lymphocyte development whether cells are destined to remain normal or become neoplastic lymphocytes. In the case of lymphocytic neoplasms, rearrangements usually occur prior to clonal expansion, and the rearranged configuration of an antigen-receptor gene therefore can serve as a unique tumor marker in a manner analogous to that of acquired genetic abnormalities.

APPLICATION OF GENE REARRANGEMENTS TO DIAGNOSIS

A monoclonal B-cell neoplasm can be distinguished from a polyclonal B-cell lymphoproliferative process through the use of DNA extraction, restriction endonuclease digestion, gel electrophoresis, and Southern blot hybridization with probes of immunoglobulin genes. For example, in a monoclonal B-cell population, one can detect a rearranged immunoglobulin heavy- or light-chain gene, because a novel restriction fragment is

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generated through the rearrangement itself (*4*). By selecting the appropriate restriction enzymes and using labeled probes of a region of an immunoglobulin probe to gene arrangement, such as the joining (J) segment to which any of the variable (V) segments might directly join, one can determine whether rearrangement has occurred. The unrearranged or germ-line configuration is found in virtually all non-B cells in the body and in very early B cells prior to rearrangement. In their germ-line state, multiple variable segments lie on the chromosome separated from the several J segments. In the heavy-chain gene, additional segments are interspersed between V and J and are known as diversity (D) elements. During rearrangement, joining of separated V, D, and J segments deletes intervening sequences of DNA from the cell. As a consequence, restriction enzyme recognition sites immediately upstream (5′) of the J region are deleted, and new restriction sites carried with the V segment are joined to a J segment (*1*). Thus a new restriction fragment size surrounding the region of joining is generated when genomic DNA is digested with the appropriate restriction enzyme and separated by gel electrophoresis. This fragment will migrate to a position distinct from the germ line.

Rearrangement of immunoglobulin genes is required for productive gene expression. Thus most monoclonal B cells show rearrangement of both the heavy- and kappa light-chain genes as most express a heavy chain and a kappa light chain. A minority of normal B cells and B-cell neoplasms (approximately 30%) express lambda light chain, and these cells have generally undergone rearrangement or deletion, or both, of kappa alleles before productive rearrangement of the lambda light-chain genes. For practical purposes, a probe of the J kappa region will detect rearrangement in most immunoglobulin-expressing B-cell neoplasms of either kappa or lambda type, because this region is deleted from both chromosomes in only about 25% of immunoglobulin lambda-expressing neoplasms.

POLYCLONAL LYMPHOCYTES

In contrast to the one or two rearrangements of heavy- and light-chain genes seen in monoclonal B-cell neoplasms, polyclonal benign B-lymphoproliferative processes and normal B-cell populations from blood in lymphoid tissue usually show no identifiable rearranged bands. Generally, no rearrangements are detected despite the B cells present in the tissue. This is a consequence of the highly polyclonal nature of B cells, which manifest many rearrangements resulting in a wide range of restriction fragment sizes spread throughout the length of the gel. None of the restriction fragments are sufficiently representative to be detected through the conventional Southern blot hybridization approach. Although the technique is sensitive and can detect as few as 1% clonal cells among the polyclonal B-cell population, it is too insensitive to detect individual rearrangements among the normal B cells in a polyclonal B-cell population. Therefore, using DNA probes and conventional Southern blots of restriction-digested and electrophoresed DNA, one can distinguish a monoclonal B-cell neoplasm from a benign polyclonal B-cell process.

T-CELL RECEPTORS

By analogy, the same approach can be used to detect clonality among T-cell neoplasms by searching for rearrangements of T-cell antigen receptor genes [reviewed in (*1, 5, 6*)]. Any of the four T-cell receptor genes, alpha, beta, gamma, or delta, may be rearranged in T cells. Most mature normal T cells express an alpha–beta heterodimer as the surface T-cell receptor, whereas few T cells express the second T-cell receptor, gamma–delta. Most T cells have successfully rearranged at least one alpha- and one beta-chain allele. For alpha-chain gene rearrangements to have occurred, the delta locus, which lies in the heart of the alpha-chain gene region on chromosome 14q11, must necessarily be deleted. However, in the case of a gamma–delta-expressing T cell, at least one delta allele is successfully rearranged, retained, and expressed, and additionally, one gamma-chain gene must also be rearranged and expressed in the same cell. Interestingly, gamma-chain genes are frequently rearranged in alpha–beta-expressing T cells. The alpha locus is difficult for one to analyze by conventional restriction fragment analysis because of the enormous size of the J alpha region that comprises approximately 85 kilobases and contains more than 50 J alpha segments. The delta locus is frequently deleted from both chromosomes in alpha-expressing T-cell neoplasms. Each T-cell receptor gene can serve as a locus for the detection of clonality of T-cell neoplasms because, as in the case of B-cell neoplasms, rearrangements occur early, i.e., before clonal expansion. Most T-cell neoplasms have been found to contain rearrangements of at least one beta-chain gene and one gamma-chain gene.

Polyclonal T-cell processes are distinguished from monoclonal proliferation by virtue of their general lack of detectable rearrangements in the beta-chain gene for the same reasons described above for immunoglobulin genes among polyclonal B cells. However, rearrangements of the gamma-chain gene can be detected even among highly polyclonal T cells (*7*). This occurs in the gamma-chain gene because the limited number of V gamma genes (<15) gives rise to only a limited number of combinations of V–J gamma joinings. In general, only eight non-germ-line restriction fragments are seen among polyclonal T cells, and these correspond to known V–J rearrangements. Therefore, one must proceed with caution when interpreting the presence of gamma-chain gene rearrangements in a diagnostic setting.

ANERGEN-RECEPTOR GENE REARRANGEMENTS IN HEMATOPOIETIC NEOPLASMS

Virtually all B-cell neoplasms contain rearrangements of the immunoglobulin heavy-chain gene regardless of the stage of differentiation of cell type, cellular morphology, or expression of immunoglobulin genes (*4, 6*). Therefore, common acute lymphoblastic leukemia has been demonstrated to be a neoplasm of precursor B lymphocytes because most cases show rearrangement of at least one heavy-chain allele, nearly one-half have rearranged a kappa light-chain gene, but most have yet to express fully assembled immunoglobulin heavy- and light-chain protein. More mature B-cell neoplasms, such as chronic lymphocytic leukemia, follicular lymphoma, large cell lymphoma, Burkitt's lymphoma, myeloma, and hairy cell leukemia display rearrangements of both heavy- and light-chain genes. Therefore, immunoglobulin gene rearrangements can serve as unique clonal markers in virtually any B-cell neoplasm.

Among T-cell neoplasms, nearly all have rearrangements of beta-chain genes, although some considered to be at the morphologic level of a mature T cell have not shown evidence of

beta-chain gene rearrangement (*4–6*). Interestingly, among T-cell lymphoblastic neoplasms, such as lymphoblastic lymphoma and acute lymphoblastic leukemia, a significant percentage (10%–20%) show rearrangement of immunoglobulin heavy-chain genes. Conversely, B-lineage lymphoblastic neoplasms frequently have rearrangements of T-cell receptor beta-chain (20%–30%), gamma-chain (45%), and delta-chain genes (75%). This phenomenon, which has been termed lineage infidelity, lineage cross-over, lineage promiscuity or bigenotypism, is found almost exclusively among lymphoblastic (immature), but not among mature lymphoid, neoplasms. Thus an individual acute lymphoblastic leukemia cell might contain rearrangements of genes of the immunoglobulin heavy-chain and T-cell receptor beta, gamma, and delta chains. It is thought that a common "recombinase," which catalyzes receptor gene rearrangements, is used for both immunoglobulin and T-cell receptor families. It is surprising that more errors in tissue specificity are not detected among mature B and T cells, either normal or neoplastic. It should also be noted that some acute myelogenous leukemias, particularly those which contain the intranuclear enzyme terminal transferase, may have antigen-receptor rearrangements. In contrast to heavy-chain gene rearrangements, those of immunoglobulin light-chain genes are exceedingly rare among T-cell neoplasms and, as lineage markers, are therefore superior to rearrangements of the heavy-chain gene.

BENIGN CLONAL LYMPHOPROLIFERATIVE PROCESSES

Although the vast majority of benign lymphoproliferative processes do not show evidence of antigen-receptor gene rearrangements, in some clinical pathologic settings, rearrangements can be detected (*1*). Rearrangements of either immunoglobulin or T-cell receptor genes have been identified in what are considered nonmalignant lymphoid tissues of patients with immunoregulatory disorders. Examples of conditions in which this phenomenon has been reported include angioimmunoblastic lymphadenopathy with dysproteinemia; congenital immunodeficiencies such as the Wiskott-Aldrich, Sjögren's syndrome, acquired immunodeficiency syndrome; and iatrogenic immunosuppression in patients following transplantation. In addition to what appeared to be oligoclonal lymphoproliferative processes occurring in such patients, these conditions are associated with increased risk of development of frank malignant lymphoma. Presumably, lymphocytes are permitted to divide excessively, resulting in the appearance of oligoclonal bands on Southern blots. Perhaps the repeated rounds of cell division put cells at increased risk of suffering a secondary genetic event, such as a chromosomal translocation, that might evoke malignant transformation and the appearance of the clinical lymphoma. From a diagnostic standpoint, it is important that one be aware of the clinical pathologic setting and understand the conditions that might account for the presence of anergen-receptor gene rearrangements.

CHROMOSOMAL TRANSLOCATIONS AS MOLECULAR GENETIC TUMOR MARKERS

The common, nonrandom chromosomal translocations associated with hematopoietic neoplasms often involve the antigen-receptor gene rearrangement process itself (*1*). For example, Burkitt's lymphoma is a B-cell neoplasm associated with a chromosomal translocation, most commonly t(8;14). Importantly, the translocation involves chromosomal band 14q32, the location of immunoglobulin heavy-chain gene. In this translocation, the c-myc oncogene on chromosome 8 is brought into close proximity to the immunoglobulin heavy-chain gene at 14q32 and is constitutively activated in this position. It is likely that the product of the c-myc gene contributes to malignant transformation. In other cases of Burkitt's lymphoma, the c-myc gene is brought into proximity to either the kappa light-chain gene on chromosome 1 or the lambda light-chain gene on chromosome 22. Numerous other examples of chromosomal translocations involving antigen-receptor gene loci in B- and T-cell neoplasms have been demonstrated at the molecular level, and in some cases genes have been shown to be deregulated as a consequence of translocation. In follicular lymphoma, a B-cell neoplasm, a chromosomal t(14;18) translocation occurs which, like Burkitt's lymphoma, involves the immunoglobulin heavy-chain gene at 14q32. However, in follicular lymphoma, a DNA segment from chromosome 18q21, termed bcl-2, is joined directly to the immunoglobulin heavy-chain region (*1, 8*).

Consistent genetic translocations associated with specific types of hematopoietic neoplasms strongly argue for their direct contribution to lymphomagenesis. Moreover, these translocations provide a type of DNA rearrangement that can be applied as a unique DNA tumor marker to detect and subclassify hematopoietic neoplasms. For example, in follicular lymphoma, a probe of the bcl-2 gene can be applied to Southern blots to detect the translocated and rearranged bcl-2 gene (*8*). By successive rehybridization of a Southern blot with bcl-2 and immunoglobulin heavy-chain J region DNA probes, the t(14;18) translocation can be demonstrated, because the immunoglobulin J and bcl-2 segments occupy the same restriction fragment. This could only have occurred through chromosomal translocation; the normal assignments of the two genes are on two different chromosomes. Therefore, cytogenetics can be performed at the molecular level instead of microscopic karotypic analysis.

DETECTION OF MINIMAL DISEASE

Advantage can be taken of the translocated region to detect minute numbers of neoplastic cells in a patient sample. Through the use of the DNA amplification technique polymerase chain reaction, synthetic oligonucleotides derived from sequences flanking the common regions of translocation, i.e., the breakpoint cluster region of the bcl-2 gene and the immunoglobulin heavy-chain J region (JH) can be used as primers to amplify the translocated and joined regions of bcl-2/JH. Because normal cells would not be expected to carry the translocation, no signal would be detected. The polymerase chain reaction has been shown to be a sensitive approach that can detect even one lymphoma cell in a patient sample (*9*). When applied in patient tissue, we have found that lymphoma can be detected when otherwise invisible to microscopy, flow cytometry, or conventional Southern blot analysis. This approach promises to be a highly sensitive means for staging, as a detector of early recurrence, and for assessing purity following bone marrow purging for autologous transplantation. Neoplasms that carry chromosomal breakpoints consistently clustered within a con-

fined stretch of DNA are amenable for polymerase chain reaction-based amplification. Many such examples of B- and T-cell neoplasms with chromosomal translocations involving antigen-receptor genes indicate that this approach may be generally applicable for diagnosis. In addition, point mutations, which might be associated with the malignant phenotype, can be detected in clinical samples with amplification of the suspected region by polymerase chain reaction and detection through sequencing or specific hybridization.

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Cytogenetics of Non-Hodgkin's Lymphoma

Ellis G. Levine[1, 2] *and* Clara D. Bloomfield[1]

ABSTRACT—In the non-Hodgkin's lymphoma (NHL), recurring cytogenetic abnormalities have been identified, and significant correlations among them and morphology, immunophenotyping, and parameters of clinical outcome have been recognized. The structural involvement of the 14q32 band is substantially more frequent than are other common abnormalities, which include del(6q), i(17q), +3, +7, +12, +18, and +21. Twenty-two recurring translocations have been identified. Almost three-fourths of all breakpoints in NHL occur at sites to which lineage-determining, transformation-related genes, or fragile sites have been mapped. Besides the well-known association of the t(14;18) (q32;q21) with the follicular histologies and t(8;14)(q24;q32) with small noncleaved cell lymphoma, several other associations between recurring cytogenetic abnormalities and morphologic subtypes have been found. Similarly, several associations between cytogenetic abnormalities and the B or T immunophenotype have been delineated. Trisomy 3 or duplications of 3p predict a favorable clinical outcome; trisomy 2 or duplication 2p and abnormalities of chromosome 17 predict a poor prognosis. Common sequential changes include a (second) 14q32 break and abnormalities of chromosomes 1 and 2. Continuing work in these areas will serve to identify more clearly those regions of the genome important to transformation, differentiation, clinical aggressiveness, and progression in NHL.—J Natl Cancer Inst Monogr 10:7–12, 1990.

Although over 1,000 cases of NHL have been examined cytogenetically (*1*), conclusions arising from the data have not always been consistent. The inconsistency may be due to several factors. First, the skill of cytogeneticists in this difficult area is not uniform. Second, karyotypes are routinely complex and consist of a broad range of numerical and structural abnormalities. Third, tumor tissue at relapse often cannot be sampled for both practical and ethical reasons, which prevents the sequential sampling of specimens and the consequent differentiation between primary and secondary abnormalities. Last, the nonuniformity of morphologic classifications, as well as the rapid evolution of immunophenotyping and of chemotherapeutic approaches in NHL, has made comparisons difficult between studies correlating cytogenetic abnormalities and other disease aspects.

Despite these shortcomings, recurring cytogenetic abnormalities have been identified, and significant correlations between these latter abnormalities and traditional descriptors of NHL have been recognized. Furthermore, the dissection of a small subset of these recurring abnormalities, primarily translocations, by molecular means has led to a substantial increase in our understanding of lymphomagenesis. Because the molecular characterization of cytogenetic changes in lymphoma is the topic of a separate discussion (unpublished), we will focus on the nonrandom chromosomal changes and their biologic and clinical associations.

RECURRING ABNORMALITIES IN NON-HODGKIN'S LYMPHOMA

Table 1 shows the specific structural or numerical abnormalities occurring most commonly in three large cytogenetic studies of NHL (*2–6*). An abnormality is listed if it occurred in 10% or more cases in a single series with the exception of the t(8;14), the rearrangement best understood at the molecular level. Observations are consistent across all three studies. The structural involvement of the 14q32 band, first identified by Manolov and Manalova (*7*), is without exception the most common in these series and many others. Although not discernible from table 1, chromosomes 1, 2, 3, and 11 are frequently involved as well; however, several different bands on these chromosomes, rather than a specific band, are affected. Despite its relative infrequency, the importance of the t(8;14) to the malignant transformation of an affected cell (*8*) clearly indicates that observational frequency and biologic importance are not necessarily correlated.

Table 2 is a list of recurring translocations found in NHL [Human Gene Mapping 10 (*9*)]; with the exception of the t(14;18), t(8;14), and t(11;14)(q13;q32), found predominantly in follicular lymphomas, small noncleaved cell lymphomas, and small lymphocytic lymphomas, respectively, these translocations are infrequently found. Recurring translocations, no matter what their frequency, have been accorded special relevance in the lymphomas since the discovery in small noncleaved cell lymphoma that the constant region of the immunoglobulin genes, i.e., the heavy-chain gene in t(8;14)(q24;q32), the λ gene in t(8;22) (q24;q11), and the κ gene in (2;8) (p12;q24), are juxtaposed to the myc gene of chromosome 8, leading to its deregulation (*8*). The subsequent identification of the putative oncogenes bcl-2 at 18q21 (*11*) and bcl-1 at 11q13 (*12*) resulting from the study of t(14;18)(q32;q21) and t(11;14)(q13;q32), respectively, are more recent examples of the molecular dissection of translocations in lymphomas. The identification of the products of these putative oncogenes and their contribution to lymphomagenesis are rapidly proceeding (*13–18*).

Koduru et al. from the Memorial Sloan-Kettering Cancer Center (*19*) combined their data on breakpoint frequency with those of the University of Minnesota series. First, they generated a random expected distribution of breakpoints based on chromosome length. Then the actual frequency of each breakpoint at a specified chromosomal region was compared with the

ABBREVIATIONS: NHL = non-Hodgkin's lymphoma; IWF = International Working Formulation for Clinical Usage; TCR = T-cell receptor.

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Table 1.—Most frequent specific cytogenetic abnormalities in NHL among three large series[a]

Abnormality	Minnesota/Australia[b]		Kristoffersson et al.[c]		5th International WS[d]	
	No.	Percent	No.	Percent	No.	Percent
14q32	172	65	23	40	101	40
t(14;18)	110	41	11	19	57	23
t(8;14)	12	5	NA		17	7
del(6q)	40	15	12	21	37	15
i(17q)	19	7	6	10	NA	
+3	30	11	8	14	22	9
+7	40	15	11	19	NA	
+12	37	14	NA		22	9
+18	28	11	9	16	NA	
+21	28	11	NA		NA	

[a] Abbreviations: 5th International WS = Fifth International Workshop on Chromosomes in Leukemia/Lymphoma; NA = not available.
[b] These 268 cases represent a combined series from the University of Minnesota and the Peter MacCallum Cancer Institute/St. Vincent's Hospital in Melbourne, Australia; 133 of them have been reported (*2–4, 10*).
[c] This series (*5*) had 58 cases.
[d] This series (*6*) presented data on 253 cases.

number expected in that region. The significance of the deviation was tested by the chi-square method. Table 3 lists the 15 bands or regions that were affected by rearrangements more commonly than expected. Most of these 15 are at sites of known oncogene locations. In fact, when all breakpoints in the combined series were considered, 68% of them were found at sites to which either lineage-determining, transformation-related genes or fragile sites have been mapped. As is the case for the breakpoints of recurring translocations, most of the identified sites have not been examined at the molecular level by anyone to determine whether a suspect gene actually plays a role in malignant transformation.

CORRELATIONS

Morphologic

Most studies have attempted correlations with histologic subtypes defined by the IWF (*20*). Investigators have reported the nonrandomness of their observations either by statistical (*3, 6, 19, 21*) or nonstatistical methods (*22–29*). In the latter studies, the numerical or structural abnormalities are individually summated, and a frequent association with a subtype is pointed out. In the former studies, chi-square analysis was used.

Table 2.—Recurring translocations in NHL

t(1;1)(p36;p11-12)	t(8;22)(q24;q11)
t(1;17)(p11 or q11;q11 or p11)	t(8;9)(q24;p13)[a]
t(1;14)(q21-q25;q32)	t(9;22)(q34;q11)
t(1;14)(q42;q32)	t(10;14)(p11;q32)
t(2;8)(q12;q24)	t(11;14)(q13;q32)
t(2;3)(q21-q23;q27)	t(11;14)(q21;q32)
t(2;5)(p23;q35)	t(11;14)(q23;q32)
t(3;14)(p21;q32)	t(11;18)(q21;q21)[a]
t(6;14)(p21;q32)	t(14;15)(q32;q15)[a]
t(8;14)(q22;q32)	t(14;18)(q32;q21)
t(8;14)(q24;q32)	t(17;22)(q11;p11)[a]

[a] These translocations were recently reported (*10*); they were not included in Human Gene Mapping 10 (*9*).

Table 4 lists the abnormalities that have been significantly associated with the individual IWF groups. Regarding those abnormalities in small lymphocytic lymphoma, trisomy 12 and 11q and 14q breaks are seen in many other subtypes. However, the presence of trisomy 12 as a sole abnormality (*3, 24*), the involvement of bands q13-14 on chromosome 11 [especially as part of the t(11;14)(q13;q32); (*30*)], and the involvement of bands q22-24 on chromosome 14 are distinctive for this subtype. Interestingly, intermediately differentiated lymphocytic lymphoma, a subtype most closely affiliated with small lymphocytic lymphoma in the IWF but with distinguishing clinical, cytologic, and immunologic features, has cytogenetic features similar to small lymphocytic lymphoma (*28*). Of the abnormalities for diffuse mixed lymphomas, trisomy 3 is closely allied with the T immunophenotype that is commonly seen in this subgroup (*4, 6, 19*).

Table 3.—Nonrandom distribution of breakpoints among 264 cases of NHL[a]

Breakpoints	Oncogenes mapped
1p34-p36	fgr
1q21-q25	sk-1, abll
1q32	
3p21	
3q29	
6q15	
6q21-q24	ros-1, myb, mas-1
8q24	myc, pvt-1
11q13	int-2, sea, hstf-1, bcl-1
11q23-24	ets1
12p12	K-ras-2
14q32	(site of Ig heavy-chain gene)
18q21	bcl-2, yes-1
18q23	
19q13	

[a] This listing was adapted from Koduru et al. (*19*).

Table 4.—Recurring cytogenetic abnormalities within histologic subgroups of NHL

Lymphoma type/IWF	Associated abnormalities
Small lymphocytic	+12, 11q, 14q
Follicular, small cleaved cell	t(14;18), del(6q)
Follicular, mixed small and large cell	+8, t(14;18), del(2q), +3/3q, 10q23-25
Follicular, large cell	+7, t(14;18), 17q21-25
Diffuse, small cleaved cell	del(8p), del(20q)
Diffuse, mixed small and large cell	+3, +5, 11p
Diffuse, large cell	+4, +7, −8, −13, +21, +X, 3q21, del(6q), 1q, 2q, 4q, 7q, 9q, 14q, 18q
Immunoblastic	+5, +18, +X, del(3p), del(5q), del(6q), 5p, 5q, 13q, 16q, 19p
Lymphoblastic	—
Small noncleaved cell	t(8;14)

Table 5.—Cytogenetic correlation with B or T immunophenotype

B[a]	T	
+12	1p[b,c]	7p15-p22[c,d]
11q	1q[c]	7q[b]
t(8;14)(q24;q32)	2p11-14[c]	7q22[c]
t(14;18)(q32;q21)	2p23-25[c]	7q32-q36[c,d]
	2q11[b]	9p21-p23[c]
	3q27[b]	10p13-p15[c]
	+3[b]	14q11-q13[b,c,d]
	4q[b]	14q32[c]
	4q21[b]	15q[b]
	6p21-23[b]	i(17q)[c]
	6q-[c]	17q21[b]
		19p13[c]
		+19[b]
		−Y[b]

[a] All are significantly associated with immunophenotype (*4, 6*).

[b] This abnormality is significantly associated with immunophenotype (*4, 6, 34*).

[c] This is among the most frequently occurring abnormalities among mature T-cell lymphoproliferative disorders (*35*).

[d] The sites of TCR are: 14q11-13 (α and δ chain), 7q32-35 (β chain), and 7p15-21 (γ chain).

Del(6q), an abnormality significantly associated with follicular, small cleaved cell lymphoma, diffuse large cell lymphoma, and immunoblastic lymphoma, rarely occurs as a sole abnormality and is more commonly found in previously treated, as opposed to newly diagnosed, patients (*2, 29*). The del(6q) is also found in a large number of nonlymphomatous neoplasms (*1*). This constellation of findings suggests that the 6q abnormality is likely to be associated with disease progression.

The t(14;18)(q32;q21) was initially associated with follicular histologies by Fukuhara et al. (*26*) and has been found in as many as 70%–80% of these histologies. Geographic differences in its frequency appear to exist, however; researchers conducting the Japanese and Russian series have identified this translocation among patients with follicular lymphomas much less commonly than have those performing the Western series (*6, 31*). The t(14;18) is seen in only 25% of the diffuse histologies; approximately one-half of the diffuse lymphoma cases with t(14;18) are known to have transformed from follicular histologies (*3, 19*). Whether all cases of diffuse lymphoma containing the t(14;18) derive from follicular histologies remains speculative. The t(14;18) is rarely seen in the high-grade histologies. On the other hand, t(8;14)(q24;q32) is the most common translocation among the high-grade histologies, specifically small noncleaved cell lymphoma (*3, 6, 22*), and is rarely seen in the low-grade lymphomas. However, similar to the t(14;18), it is not uncommonly found in those of intermediate grade (20%), particularly diffuse large cell lymphoma. The human immunodeficiency virus-associated lymphomas, virtually all high grade, commonly contain the t(8;14) (*32*).

Immunologic

Attempts by researchers to link specific cytogenetic abnormalities with immunophenotypic groups of NHL have involved both statistical and nonstatistical methods (*4, 6, 33–35*). Statistical methods have been restricted to large studies including both B- and T-cell lymphomas.

Table 5 lists the abnormalities associated with these two immunophenotypes. Cases of adult T-cell leukemia/lymphoma were included in the compilation of this table; however, because there are only minor cytogenetic differences between human T-cell lymphotropic virus-I positive and negative lymphomas, their contribution to the data is not segregated. The preponderance of abnormalities linked with T-cell as opposed to B-cell NHL is in large part due to the relatively much smaller number of T-cell cases in most series and the complexity of many T-cell karyotypes; this situation favors the finding of significant deviations from the expected among the smaller group in the chi-square method.

The t(8;14) and t(14;18) were once thought to be specific to B-cell malignant diseases, a conclusion that has been challenged by recent reports (*36, 37*). However, many of these cases may represent the pseudo-T lymphoma described by Jaffe et al. (*38*), a B-cell lymphoma so heavily infiltrated with T cells that its true immunophenotype can be falsely deduced. The specificity of these two translocations therefore remains unsettled.

Not surprisingly, the sites of the TCR genes are among those most commonly disrupted in T-cell NHL. However, the frequency of involvement of the sites of TCR genes in T-cell cases (4%–7%) is low (*34*) compared with that of the 14q32 band containing the immunoglobulin heavy-chain gene (12%–45%). Indeed, besides the 14q32 band, both arms of chromosome 1, 2p, and 6q are generally more heavily involved in T-cell lymphomas than are the TCR gene sites (*35*).

These data again point out that frequency of occurrence may have little to do with biologic importance. The identification of the putative oncogenes tcl-1 (*39*), tcl-2 (*40*), and tcl-3 (*41*), as well as the deregulation of myc (*42*), resulting from the molecular characterization of breakpoints in the infrequently occurring malignant T-cell abnormalities inv(14)(q11q32), t(11;14)(p13;q11), t(10;14)(q24;q11), and t(8;14)(q24;q11), respectively, serves to amplify this fact.

Only one research group (*4*) has attempted to correlate cytogenetic abnormalities with surface or cytoplasmic immuno-

globulin, or with the presence or absence of antigens, or with their combinations recognized by monoclonal antibodies. Several significant associations were identified. The correlation of cytogenetic abnormalities with combinations of antigens representing a distinct stage of cell maturation is an exciting direction for future studies. Whether a specific stage of differentiation "permits" chromosomal breaks to occur, or whether cytogenetic breaks ultimately determine stage of differentiation is presently unclear. If the former hypothesis is true, one might speculate that transcriptionally active areas are those most prone to breakage and rearrangement. Mapping of these break sites would therefore provide information regarding the transformation and differentiation of lymphocytes.

Although correlation of cytogenetic abnormalities with the same parameter, e.g., the morphologic groups defined by the IWF, has been important in allowing the collation of data from several studies, the inflexibility of this type of analysis does not permit the identification of cytogenetic subgroups not fitting the commonly used parameters. That such unique cytogenetic subgroups exist in NHL has been recently observed by Kaneko and colleagues (*43, 44*). They have described a childhood large T-cell lymphoma containing the t(2;5)(p23;q35) that mimics the morphologic appearance of malignant histiocytosis and a T-cell lymphoproliferative disorder commonly containing trisomy 3 or 5 that has features of angioblastic lymphadenopathy with dysproteinemia.

Correlations With Clinical Outcome

Several reports have correlated karyotypic characteristics or specific abnormalities with measures of clinical outcome. With few exceptions, however, these studies have included patients whose karyotypes were first examined at diagnosis or relapse (*24, 27, 45–47*). The prognostic relevance of cytogenetic changes should be confined to patients whose karyotypes have been examined at diagnosis, because karyotypes first examined at relapse or following treatment may contain evolutionary cytogenetic changes unrepresentative of karyotypes at diagnosis. Two large studies have been reported that satisfy these conditions.

Among 68 newly diagnosed patients with NHL, Levine et al. (*48*) found that the presence of any normal metaphases predicted an increased response rate and a longer survival. Whether the normal metaphases represented reactive T cells or malignant lymphocytes lacking microscopic cytogenetic changes could not be discerned. Alternatively, the presence of a break at 1p32 tended to result in a shorter survival. Among patients with follicular lymphomas, the presence of an increasing number of normal metaphases again predicted for a higher complete response rate and a longer survival. On the other hand, abnormalities of 17p or q predicted a shorter survival; no two rearrangements involving chromosome 17 were the same. Two other groups of investigators (*25, 49*) have also found abnormalities of chromosome 17 to have adverse prognostic significance. Among diffuse large cell and immunoblastic lymphomas, abnormalities of 2p predicted a longer survival; no 2p abnormalities were the same. Although a multivariate analysis was not conducted, known prognostic factors were evenly distributed among the cytogenetic groups demonstrated to be of clinical importance.

Yunis and associates (*25*) correlated cytogenetic abnormalities with clinical outcome among 74 newly diagnosed patients with NHL. All had large cells as a morphologic characteristic. Of the 20 that were follicular lymphomas, 8 were mixed small cleaved and large cell and 12 large cell, and of the 54 that were diffuse, 13 were mixed, 22 large cell, and 19 immunoblastic. Among the patients with diffuse histologies, trisomy 3 or dup 3p predicted a favorable course, and trisomy 2 or dup 2p and −17 or del 17q, an unfavorable course. The best-fitting multivariate model contained dup 3p, dup 2p, del 17q, and T-cell phenotype; the latter phenotype had a negative impact on survival. Similarly, among the 20 patients with follicular lymphomas, dup 3p or +3 and dup 2p or +2 was associated with a favorable and unfavorable outcome, respectively; however, the number of patients with these abnormalities was too small for meaningful statistical comparisons. Among two subgroups of patients, those with B-cell nonimmunoblastic diffuse lymphoma and those with follicular lymphoma, the presence of a bcl-2 rearrangement was associated with a poor response to treatment.

Correlations With Clinical Sites of Disease

This area is largely unexplored. In the study of Levine et al. (*48*), gastrointestinal involvement was associated with abnormalities of the 11q13 band and meningeal involvement with trisomy 11 and del (6) (q23) in the small number of patients with involvement of these sites. No association has been identified between cytogenetic characteristics and either extent of disease, i.e., stage, or the presence of A or B symptoms (*48*).

SEQUENTIAL CHANGES OF THE KARYOTYPE

The distribution of additional and evolutionary cytogenetic changes among the acute leukemias and chronic myelogenous leukemia is nonrandom and suggests that chromosomal regions are responsible for disease progression (*50, 51*). In addition, subsequent chromosomal changes among these leukemias seem dependent on a primary chromosomal defect, defined as an abnormality found frequently and often alone in a disorder or one of its subtypes. Investigators' attempts to identify recurring additional abnormalities in NHL have proceeded in two ways. In the first, sequential karyotypes are examined in patients who have had two or more biopsies over the course of their disease. In the second, recurring cytogenetic changes have been identified among karyotypes considered to have a primary cytogenetic alteration, i.e., either t(14;18) or t(8;14).

Two groups have examined evolutionary changes in NHL using the former approach. Levine and co-workers (*52*) studied 21 patients with a broad range of histologic subtypes. The most frequent newly acquired abnormality was a break at 14q32 (5 of 16 patients with karyotypic evolution). Chromosomes 1 and 2 were also frequently involved by acquired changes, but no particular band or region was repeatedly affected. The t(14;18), present in the initial karyotypes of 10 patients, was retained in all instances, thereby suggesting the primary importance of this cytogenetic rearrangement. Of additional interest, the 4 patients with acquired deletions of all or part of 17p had a significantly shorter survival from the date of its identification than the remaining patients without 17p deletions at the time of sequential biopsies. Last, karyotypic change did not predict histologic change; the reverse was also true.

Sanger et al. (*53*) sequentially examined the karyotypes of 12 patients from 5 morphologic subgroups of NHL. Similarities to the above study included retention of the t(14;18) in 6 of 6 cases

and the lack of association between karyotypic evolution and histologic transformation. In this series, an abnormality of chromosome 1 was the most commonly required alteration.

While examining karyotypes with a t(14;18), several investigators have identified recurring additional abnormalities and assumed that they represent secondary changes (*6, 24, 54–56*). The most frequent additional abnormalities, occurring in 10%–40% of such karyotypes, include trisomies of X, 7, 12, 18, and 21; deletions of 6q; and either isochromosome 17q or trisomy 17. Trisomy 7 or a duplication of its q arm is more commonly found in diffuse, as opposed to follicular, morphologic subgroups among patients with t(14;18) (*54*). Furthermore, these same abnormalities of chromosome 7, as well as trisomy X, are found significantly more often as additional abnormalities in karyotypes containing t(14;18) than in those containing t(8;14) (*6*). Fukuhara and associates (*55*) have claimed that an extra 18q− is a more common abnormality among Japanese patients with t(14;18) than among those in non-Japanese series.

Among patients with t(8;14), a duplication of material on the 1q arm, usually between bands 1q21 and q32, has been the most common additional abnormality (*6, 57*). It occurs significantly more often in karyotypes containing a t(8;14), as opposed to a t(14;18) (*6*).

SUMMARY

Although a considerable number of karyotypes have been examined in NHL, their significance remains unclear due to the innate complexities of both the cytogenetic abnormalities and the disease itself. Nonetheless, associations between recurring cytogenetic abnormalities and morphology, immunophenotype, and parameters of clinical outcome have been made, and secondary karyotypic changes have been delineated. Continuing work in these areas will serve to identify more clearly those regions of the genome important to transformation, differentiation, clinical aggressiveness, and progression in NHL.

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A Staging of Lymphomas: Practical Thoughts on Impractical Practices

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ABSTRACT—Staging of lymphomas represents a discipline different from the staging of other cancers. In lymphomas, the purpose of staging rests more on the establishment of consistent standards by which we can evaluate changes in treatment programs, rather than a means of deciding on an appropriate treatment for an individual patient. In comparing treatments from different times and centers, one should exercise care to ensure the true comparability of the study groups. Frequently, errors are encountered in the comparison of surgical versus clinical staging. Other pitfalls occur when new, more sensitive diagnostic tests result in the clinical upstaging of selected groups leading to the improvement in subgroups but not affecting the treatment results for the group as a whole.—J Natl Cancer Inst Monogr 10:13–17, 1990.

Many factors influence the decisions that are made by physicians concerning cancer treatment. Probably the most important of these is staging. Among most physicians today, some confusion persists about the process and purpose of staging in lymphomas. Therefore, we offer these thoughts with the idea that they will help clarify various issues on the subject.

WHAT IS STAGING?

Staging is the process of defining the extent of a neoplastic disease within a given patient in regard to its anatomic spread. For most neoplastic diseases, staging consists of assigning a letter or numerical code that represents progressive extent of disease. The information that permits this categorization is usually obtained by physical examination, diagnostic radiologic workup, and in some cases, surgical procedures. It typically summarizes a number of key factors about the malignant process: 1) the size or volume of the primary tumor, 2) the extent of local invasion, 3) nodal involvement, and 4) the presence of distant metastasis.

Most modern staging systems are based on the TNM (tumor, node, metastasis) system, with the first major criterion being based on the size of the primary lesion. This is *not* the case for the lymphomas. The Ann Arbor staging system (*1*) is the most commonly used and, although originally developed for Hodgkin's disease, it is also generally used for most of the non-Hodgkin's lymphomas. This system is based primarily on the gross distribution of lymphatic involvement with respect to the diaphragm (table 1), and for advanced stage, the presence of organ involvement. No consideration is given to tumor size. This is not an oversight, but rather a deliberate, yet tacit acknowledgment that in lymphoma patients, spread to other sites takes prognostic priority over mere size. Yet size is still an important prognostic consideration *within* any single staging category (*2–4*). Large tumors do not necessarily indicate advanced stage for the lymphomas. The diaphragm is used as a reference point simply because most patients have a diaphragm, and because this structure conveniently splits the torso into two nearly equal volumes that represent approximately the largest conventional field size that can be achieved with modern megavoltage radiotherapy equipment.

In any neoplasm, the individual categories of stage, either number or letter, that are used for grouping patients are based on clinical and physical determinants of operability, resectability, and radiologic or clinical diagnosis. In the process of grouping diverse patients, each of the groups acquires an associated prognosis. Typically, there is a correlation between the degree of invasion or size and the outcome of the patient. On the other hand, we have no a priori reason to presume that each stage must have a different prognosis, because the anatomic definitions of stage are fundamentally arbitrary.

In some cancers, such as carcinoma of the cervix, staging is an invaluable tool because the appropriate treatment and prognosis are tightly correlated with the stage (*5*). In lymphomas there is a range of prognosis depending upon many factors, some of which may not be recognized. The prognosis for any one group represents not only the blending of several populations within that staging category, but also the numerical frequency of what type of patient is most typically seen within that staging category. For example, a patient with massive mediastinal Hodgkin's disease may represent stage I-A if involvement does not extend beyond the mediastinum. Even so, the prognosis of this I-A patient is markedly different from that of the typical I-A patient. Stage II can also include an enormously heterogeneous group, which can represent involvement from 2 to 12 separate lymphatic sites, based on the arbitrary reference of standard lymphatic regions set forth in the Ann Arbor system (fig. 1). Stage III is even more heterogeneous than is stage II. The patient who has an isolated left supraclavicular node of Hodgkin's disease and who, on staging laparotomy, also has an isolated involved para-aortic node, represents stage III_2A Hodgkin's disease. Nonetheless, in our experience, with only two sites of involvement, this is a highly favorable patient, although an uncommon one. Another example of marked heterogeneity within staging groups occurs with small cell non-Hodgkin's lymphoma: Patients within groups vary dramatically in prognoses depending on their histopathologic subclassification (*7*). Thus it is extremely important that physicians fully recognize the enormous heterogeneity that exists *within* any one staging category. Contrast this again with the case of cervical cancer: A stage III-B patient with side wall involvement is representative of a much more homogeneous population with more similar prognoses.

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Table 1.—Modified Ann Arbor Staging Classification

Stage	Definition
I	Involvement of a single lymph node region (I) or a single extralymphatic organ or site (I_E).
II	Involvement of two or more lymph node regions on the same side of the diaphragm (II) or localized involvement of extralymphatic organ or site and of one or more lymph node regions on the same side of the diaphragm (II_E). Optional recommendation: indication of number of node regions involved by subscript number (e.g., II_3).
III	Involvement of lymph node regions on both sides of the diaphragm (III), possibly accompanied by localized involvement of extralymphatic organ or site (III_E), or by involvement of the spleen (III_S), or both (III_{SE}).
IV	Diffuse or disseminated involvement of one or more extralymphatic organs or tissues with or without associated lymph node enlargement. Reason for classification of patient as stage IV identified further by defining site by symbols.

WHY DO WE STAGE PATIENTS?

In the present era of patient treatment protocols that are frequently based on disease stage, we must recognize that the process of staging provides a mapping of the extent of a patient's disease. The main purpose of this process is to allow meaningful comparisons of results among institutions, among different eras, and with different treatments. Because most studies of treatment results are retrospective and not prospectively randomized, we rarely have appropriate controls that permit realistic and accurate interpretation of a new treatment. It is this process of staging that allows physicians to interpret the results by comparing similar kinds of patients with respect to their overall outcome. This is the most important reason for staging patients.

Because there is typically (but not necessarily) a prognostic correlation from stage to stage, staging is frequently useful for estimating the prognosis of a patient and allowing that prognosis to influence the decision about how aggressive or conservative treatment should be for a particular patient. Again, however, we must emphasize that within any individual staging category, an enormous heterogeneity of prognosis is present, depending upon the tumor and patient characteristics, some of which are not yet fully recognized.

Ideally, any staging system should be useful in clinical research and standard medical practice. It should be related to prognosis, although it is not absolutely necessary for major differences to occur among all the different groups. An optimal staging system should be objective, simple to learn and, most especially, easy to reproduce. Many individuals believe that because there is ideally a prognostic correlation among stages, the staging classification should reflect and include *all* the relevant prognostic features. Great care should be exercised on this point, because although we know of many prognostic factors, we need to be aware of many others. Our expanding knowledge of the molecular biology of these tumors is likely to reveal more of these prognostic factors. Even so, these are not anatomic features, and staging of neoplasms is the process of defining the extent of the disease in *anatomical* terms (with the obvious exception of B symptoms). Staging across the field of neoplasia relies on the use of anatomic groupings of similar patients. The addition of biochemical measurements confuses the anatomic nature of the staging definitions. Furthermore, use of these new techniques to determine stage confounds the reproducibility of stage by the introduction of differences in laboratory techniques at various institutions and the availability of tests outside a given research institution.

PITFALLS IN STAGING OF PATIENTS

It is usually assumed that all patients who have early stage disease inherently have a better prognosis than the patients with late-stage disease. This is not always true, as is observable in certain patients with nodular lymphoma, in whom even bone marrow involvement does not imply a major reduction in survival (*8*).

Another potential problem is the assumption that so-called early stage disease represents purely a local or local–regional problem, which needs only local treatment. Furthermore, confusion and disagreement occur frequently among various oncologists who might be asked to stage a patient with a hypothetical presentation of lymphoma. Some oncologists will use the "E" category to represent limited extralymphatic extension, whereas

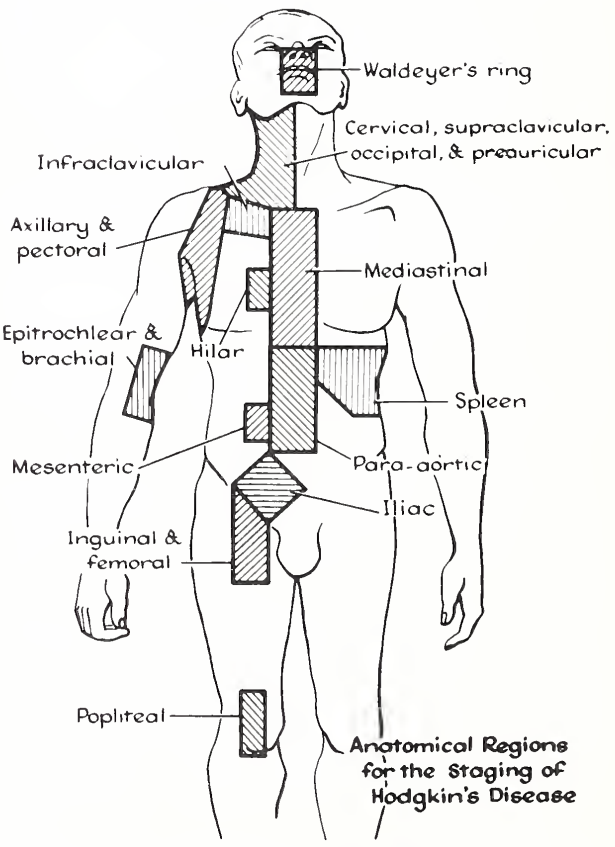

FIGURE 1.—Schematic representation of the regional areas used in the staging of Hodgkin's disease (*6*). Figure is reproduced with permission of one of the authors and the publisher.

others will interpret the same presentation as disseminated disease stage IV. Frequently, the difference among oncologists correlates with the subspecialty training, i.e., medical oncologists will often classify someone as stage IV because they believe the patient should be treated with chemotherapy, whereas radiation oncologists may classify the patient as an E because they see the problem as focal extension and not true dissemination. This kind of dilemma will not be resolved by any simple discussion; the point is that simply defining the patient's disease in anatomic terms (i.e., what we call staging) is sometimes ambiguous. Staging is a separate discipline from determining the appropriate treatment for an individual patient. In no way does it necessarily follow that the disease stage must dictate the treatment for a patient. It is this fact that provides room for the lymphomatologist and separates the treatment of lymphomas from other cancers for which stage is more highly correlated to an appropriate treatment (although, even with other forms of cancer, factors other than stage, such as performance status and treatment morbidities, enter into the decision-making process for determining appropriate treatment).

Under the auspices of clinical research, many protocols are predicated upon the stage of the patient, allocating the patient to one or two alternatives of treatment that are designed for that specific stage. The reader should be aware that this is fundamentally a political decision, rather than a medical one. It is difficult, if not impossible, for one to weigh all the various contingencies that may be important in determining the most appropriate treatment for any individual patient. Indeed, the contingencies are so numerous that anyone would find it difficult to design prospectively randomized trials with sufficient numbers of patients to obtain valid results. It is precisely the ability to focus on something as simple as stage and to neglect all the other factors that allows us to conduct prospectively randomized trials at all. The assumption is that by our randomizing patients by stage, the other factors that are important prognosticators will be averaged over the population studied. Although disease stage may be the most important single prognostic variable, its importance should not be exaggerated when treatment decisions are made. We abhor the thought of a "Betty Crocker mind set" in determining treatment options for individual patients not on research protocols.

Another pitfall in staging concerns the not-so-subtle distinction that exists between clinical and surgical staging. Patients who have undergone surgical procedures to define the anatomic extent of their disease obviously present far more definitive information than can be determined by clinical examination alone. A comparison of clinically staged patients with pathologically staged patients is simply invalid. Although the anatomic definitions of stage may be the same for the surgically and clinically staged patient, the method of defining the stage is so different as to negate any kind of direct comparison. Yet such a comparison occurs when investigators attempt to document the percentage of patients whose stage was changed by a surgical procedure. This comparison is invalid, because a presumption is made that the two populations are similar. Also, the fact is overlooked that merely the patients' ability to undergo the surgical staging would dramatically differentiate the two populations. This is less of a problem in patients with lymphoma than in those with many of the other solid tumors; however, it is still an issue that requires attention and considerable skill when results from various studies are interpreted.

A corollary to the pitfall of comparing surgical staging with clinical staging is the phenomenon of stage migration, secondary to improved accuracy of pretreatment evaluation, especially when some new technology or surgical procedure is introduced within the staging process. This phenomenon was originally described by Bush (*9*), and was later popularized as the "Will Rogers phenomenon" (*10, 11*). The changes introduced may be subtle because the anatomic definition of the stage does not change, but the sensitivity and specificity for determining the presence of disease may be altered. This was clearly seen with the advent of computed tomographic scans of the abdomen done with oral and iv contrast that has allowed detection of involvement in this area that previously was possible only through laparotomy. Another example exists in Hodgkin's disease, when surgically staged patients are compared with clinically staged patients: Individuals who are found to have occult disease at exploratory laparotomy are recategorized as having advanced disease. This results in more appropriate treatment of both the advanced and the early stage group. Treatment outcome of the early stage group is improved by selection of those patients with more advanced disease, and that of the advanced stage group is improved over historical controls by inclusion of patients with minimal disease beneath the diaphragm. This problem can also arise when comparisons are made between one era's stage X, and another era's stage X, for they may represent two drastically different groups of patients, with dramatically different prognoses. In this way, the improvement in patient outcome can be demonstrated for each stage, and the overall results for all patients remain unchanged (*10*). This fallacy of invalid comparison of stage can lead to conclusions about benefits of newer treatments that may not be real. Another cause of stage migration can occur when differences occur in interpretation of diagnostic procedures, such as lymphangiograms, that may lead to improper conclusions about studies done at different institutions conducted at the same time with similar protocols. Caution must always be exercised and care given for assurance that similar patient populations were studied and similar diagnostic criteria were used in the comparison of any clinical studies.

RECOMMENDATIONS FOR STAGING OF LYMPHOMA

Having discussed staging in general, we include in table 2 our recommendations for the staging of lymphomas. Obviously, many specific procedures are considered essential for staging; in addition, more may be helpful in the staging of individual patients.

It is still our view that the lymphangiogram is probably the most beneficial procedure for evaluating retroperitoneal lymphadenopathy. Our reasons for this are as follows:

1) The lymphangiogram is the most sensitive radiologic procedure for assessing early nodal involvement because of its ability to assess the altered internal architecture of the node radiologically; computed tomography scan only shows the criterion of size. A recent review (*12*) from Stanford stated that lymphangiograms compared with computed tomography scans had greater sensitivity (100% vs. 86%) and overall accuracy (91% vs. 82%).

Table 2.—Procedures used for staging lymphoma

Procedure	Indications	Diagnostic yield
History	All patients	Night sweats with fever, weight loss >10% in the 6 mo prior to admission
Physical examination	All patients	Involved lymph nodes, extranodal sites, pericardial rubs, pleural effusions, hepatosplenomegaly
Chest x ray	All patients	Mediastinal, hilar disease, pericardial effusion or mass, pleural effusion, cardiophrenic adenopathy
Lymphangiogram	All patients, unless contraindicated by pulmonary dysfunction or allergy	Periaortic, common iliac, external iliac, or femoral lymphadenopathy
Computed tomography scan of the chest	Suspected mediastinal, hilar, or pulmonary parenchymal disease	Mediastinal, hilar, or pulmonary parenchymal disease
Liver/spleen scan	All patients	Hepatic or splenic involvement
Bone scan	Symptomatic or elevated alkaline phosphatase	Osseous involvement (correlate with bone x rays)
Computed tomography scan of the abdomen	Equivocal lymphangiography or liver/spleen scan	Enlarged lymph nodes, splenomegaly, filling defects in liver or spleen
Bone marrow biopsy	All patients	Marrow involvement with lymphoma
Gallium scan (optional)	General screening for areas of lymphomatous involvement	Increased uptake in areas of adenopathy
Laparotomy	*See* text	Pathologic evidence of subdiaphragmatic disease

2) For follow-up evaluation, it is frequently difficult for one to obtain the same level on sequential computed tomography studies, making the assessment of size problematic.
3) Lymphangiograms with follow-up films result in lower doses of radiation than do sequential computed tomography studies.
4) The cost of repeated scans is more expensive than lymphangiograms with follow-up flat plates.
5) The lymphangiogram is frequently helpful in defining specific nodes for biopsy when surgical exploration is planned.
6) The lymphangiogram is of extraordinary value in setting up radiation portals, if the patient is to be treated with radiation to the retroperitoneum.

Computed tomography or magnetic resonance imaging can add enormously to our detection of nodes beyond the retroperitoneum. These diagnostic procedures therefore complement the lymphangiogram invaluably.

As far as the staging laparotomy is concerned, we want to emphasize that it is an elective procedure that has its greatest role to play in patients who are being considered for irradiation alone. It is this group of patients for whom surgical findings may define more clearly the most appropriate treatment. If a staging laparotomy is to be done, it is important that it be undertaken after the physicians responsible for the patient and the surgeon have reviewed the case and films jointly and have agreed upon exactly what the surgical objectives are.

CONCLUSIONS

We hope that the reader appreciates that staging for any disease, especially lymphoma, is far from perfect, and that a perfect system has not been defined. Nonetheless, clinicians must use this imperfect tool while acknowledging its deficiencies. Currently, it is the best tool that we have, and conclusions about clinical studies will depend on its proper use, as well as on subsequent analyses of research results by statisticians with their computers and multiple independent regression analyses. At the same time, the conclusions drawn from such analyses will have to be interpreted by clinicians carefully, and the quality of the data on which those conclusions were formulated will have to be judged accordingly. Such an evaluation, based on common sense and careful interpretation of the procedural methods and data, may be more important than the mystical *P* value.

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Treatment of Hodgkin's Disease

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ABSTRACT—The available data support the hypothesis proposed by Smithers that Hodgkin's disease appears to be a systemic disorder of the lymphatic system. Standard treatments have been developed that cure approximately 70% of all patients who present to most institutions. Physicians should know the treatment approaches before proceeding with staging, because intelligent use of the best available treatment often obviates the need for staging laparotomy. At present, it is best that either chemotherapy or radiotherapy be used alone, except in patients who have massive mediastinal disease and for whom combinations of radiotherapy and combination chemotherapy are superior. Despite the long series of clinical trials conducted over the past two decades, no combination of four drugs has improved the results obtained with the original mechlorethamine–vincristine–procarbazine–prednisone program, when it is given in sufficient doses. It has been assumed that drug resistance of a specific type was the major reason for treatment failure. Attempts by physicians to overcome drug resistance, using alternating cyclical non-cross-resistant combination chemotherapy, have thus far not proved this approach to be superior to the use of a four-drug combination in full doses, and call into question this approach to testing the Goldie-Coldman hypothesis. Dose intensity has been a poorly controlled variable in virtually all clinical trials in Hodgkin's disease, and inadequate dosing may be the prime reason for treatment failure. This point has been highlighted by recent excellent results with marrow-ablative, high-dose chemotherapy and autologous bone marrow transplantation support for patients with very advanced "drug-resistant" disease. Investigators are now attempting to improve dose intensity by using more concentrated versions of standard drug combinations with colony-stimulating factors for support.—J Natl Cancer Inst Monogr 10:19–28, 1990.

It has been 19 years since the first report that Hodgkin's disease was curable by drugs, and since then we have learned a great deal about how to approach the treatment of this disease (*1*). Here we review current approaches to treatment in the light of new data on alternating cyclical chemotherapy and salvage treatment programs against the backdrop of some comments on the natural history of the disease.

ABBREVIATIONS: NCI = National Cancer Institute; MOPP = mechlorethamine–vincristine–procarbazine–prednisone; RDI = relative dose intensity; MVPP = mechlorethamine–vinblastine–procarbazine–prednisone; ABVD = doxorubicin–bleomycin–vinblastine–dacarbazine; CABS = cyclohexyl nitrosourea–doxorubicin–bleomycin–streptozocin.

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THE REED-STERNBERG CELL AND THE MODE OF SPREAD OF HODGKIN'S DISEASE

A unique feature of Hodgkin's disease is that the malignant cell and its mononuclear counterpart make up the minimal portion of any palpable or visible tumor mass. At any given time, for any given stage of the disease, therefore, fewer malignant cells are present compared with other lymphomas or solid tumors of viscera. This fact may account for the sensitivity of Hodgkin's disease to drugs. We are still not sure of the origin of the Hodgkin's cell. What little is known of its biology is reviewed elsewhere in this volume. At the moment, the best candidate for the cell is an antigen-presenting cell, the interdigitating reticulum cell, located in the paracortex of the lymph node, although some more recent data on the configuration of the immunoglobulin genes suggest an origin from B cells. The natural history of the disease suggests a cell that normally dwells in lymphoid tissue and has a preference for metastasis to such tissue. In fact, it was because of this pattern of behavior that the late Henry Kaplan said that in order to learn how to treat Hodgkin's disease, you have to learn to think like a Reed-Sternberg cell (*2*). That early stages are curable by radiotherapy and increasingly curable as field size increases had a strong influence on Kaplan's thinking (*3*).

He believed that the disease began unicentrically and spread by relentless involvement of contiguous lymph node areas, only spreading hematogenously late in its natural history. When staging data began to reveal certain inconsistencies in this hypothesis, such as the common association of left supraclavicular and para-aortic node–lymph node involvement without mediastinal involvement, Kaplan hypothesized that the retroperitoneum was populated by cells that spread retrograde down the thoracic duct from the left supraclavicular nodes. He believed this occurred because of increased pressure associated with obstructive lymph node involvement. Radiation fields were designed to encompass the primary site of involvement and contiguous node areas. This led Kaplan to test total lymph node radiation therapy, a radical and successful innovation in its time. The concept was extended further when it was reported that extensions of Hodgkin's disease into adjacent viscera from involved lymph nodes could be successfully treated by extending the radiation field to encompass the appropriate portion of the involved organ. Staging classifications were modified to reflect this by adding the subscript "E" to distinguish visceral involvement of this type from disseminated involvement (*4*).

The Kaplan hypothesis holds sway even today and yet it is severely flawed. First, too many skip areas are unexplained by retrograde flow. In addition to the mediastinal skip, it is not uncommon for one to see bilateral, axillary, lymph node involvement and bilateral neck node involvement with no intervening nodes. Second, lymphatics have valves to prevent reverse flow and, even when obstruction is noted on lympho-

grams, collaterals develop in the direction of normal lymphatic flow. The most telling observation, however, comes from Kaplan's studies on staging with laparotomy. In those studies, splenic involvement was common even in patients with normal-sized spleens. Because the spleen has no afferent lymphatics, this could only occur through early hematogenous invasion.

At the time Kaplan proposed his hypothesis, Sir David Smithers (*5*) provided a different explanation that more nearly fits the available data. Smithers proposed that Hodgkin's disease was a disease of the entire lymphatic system and, although it tended to occur unicentrically, it could occur in a multicentric fashion. When it begins at a single site, he proposed, the malignant cells spread from the lymph node of origin, through adjacent lymphatics to other lymphoid-bearing tissue, in the direction of flow, with early access to the blood stream. Of particular note is his proposal that the Hodgkin's cell, regardless of how and where it traveled, grows preferentially in lymphoid tissue. This hypothesis explains most of the observations in Hodgkin's disease, in that the disease often appears unifocal and spreads by contiguity; skip areas can be explained by preferential sites of involvement of the circulating Hodgkin's cell, including involvement of the spleen. That radiotherapy destroys not only the malignant cells in the treatment field but also the sites that the cells preferentially inhabit must be the reason it cures patients with Hodgkin's disease, despite cell migration via the blood stream. The assumptions behind the Smithers' hypothesis certainly seem reasonable in light of the subsequent discovery of growth-promoting lymphokines that would be produced in largest amounts in spleen and lymph nodes and in cell-adhesion molecules that could account for selective homing of blood-borne cells. Smithers hypothesized that Hodgkin's disease is a systemic illness at its outset. This gives credence to the concept of physicians using systemic treatments in early stage disease that are effective in advanced stages, without the need for radiation therapy.

At the moment, it appears that when we design treatments for Hodgkin's disease, we should learn to think like Sir David Smithers' Reed-Sternberg cell, a cell unique to lymph nodes that travels by both lymphatics and blood vessels, but prefers to inhabit lymphoid tissue until it becomes less stable. It is a cell that evinces extraordinary sensitivity to radiotherapy and combination chemotherapy and, perhaps, in its early history, to destruction of its normal habitat.

TREATMENT BY STAGE

Before commenting on new approaches to treatment, we need to review standard approaches to the treatment of Hodgkin's disease.

Staging of Hodgkin's disease is covered elsewhere. However, one point needs to be reemphasized: Clinicians should first know how they intend to treat Hodgkin's disease on a stage-by-stage basis before embarking on a staging workup. Some approaches to treatment obviate the need for certain staging procedures such as laparotomy (*4*).

Standard approaches to treatment by stage are shown in table 1 for previously untreated patients and in table 2 for patients who have failed primary treatment. If one uses the approaches listed in table 1, then staging laparotomy is not indicated under the circumstances outlined in table 3 (*6*). Regardless of stage, the use of combination chemotherapy and radiotherapy is indicated only in patients with massive mediastinal involvement. Salvage treatment of radiation failures with chemotherapy is so successful that, in most studies, long-term survival of patients with early stage disease is not improved when combined modality approaches are used as primary treament. Too often physicians use combined modality therapy in early stage disease without considering the fearsome consequences of long-term toxic effects from such an approach.

Although some confusion remains about the treatment of III-A disease, the results of almost all studies indicate that combination chemotherapy is superior to the use of radiotherapy alone and, at the least, is equivalent to the use of combination chemotherapy and radiotherapy. Because the latter approach only adds to the toxic reactions, there is no reason for physicians to recommend it.

Table 1.—Treatment by stage

Stage	Description	Treatment
I, II	No massive mediastinal involvement	Radiotherapy alone
III, IV	No massive mediastinal involvement	Combination chemotherapy alone
Any stage	Massive mediastinal involvement	Chemotherapy followed by radiotherapy

Table 2.—Salvage treatment[a]

Categories of treatment	Frequency, %	Treatment approach
Radiotherapy	40%	—
Primary failure	<5	Chemotherapy
Relapse	±20	Chemotherapy
Chemotherapy	60%	
Primary failure	20	IC ± total-body irradiation + autologous bone marrow transplantation or crossover
Relapse		
<1 yr	15	IC ± total-body irradiation + autologous bone marrow transplantation or crossover
>1 yr	15	Re-treat with same regimen
Relapse after multiple exposures to chemotherapy and radiotherapy		IC ± total-body irradiation + autologous bone marrow transplantation

[a] With standard treatment, 40 patients will fail for every 100 treated, 20 will be resistant, and 20 still sensitive to chemotherapy. IC = intensive chemotherapy.

DIRECTIONS IN THERAPEUTIC RESEARCH

In the past 10 years, three general trends in therapeutic research in Hodgkin's disease have been noted: 1) researchers'

Table 3.—Hodgkin's disease

Staging laparotomy not required for:
Stages III, IV-A, IV-B
Patients with massive mediastinal involvement
Isolated involvement
Axillary[a]
High neck[a]
Mediastinal
Inguinal
Elderly or medically infirm patients, or both
Pediatric patients who will receive combined modality treatment
Women with clinical stage I disease[b]
Men with clinical stage I disease and mediastinal involvement only, lymphocyte predominance, or interfollicular histology[b]

[a]Laparotomy is not indicated if ipsilateral scalence node biopsy is negative.

[b]Laparotomy is not indicated according to Leibenhaut et al. (*6*).

attempts to improve existing treatment programs by decreasing drug toxicity, 2) the design of treatments to overcome drug resistance, and 3) treatment designed to capitalize on the extraordinary sensitivity of the Hodgkin's disease cell to chemotherapy.

The great preponderance of clinical investigators have explored the development of less toxic regimens. Although this is a noble cause, it presumes satisfaction with the current results. Substantial numbers of patients with Hodgkin's disease are not yet curable, so the major effort should go toward improving cure rates. Various standard drug combinations allow physicians to select treatments suitable for the specific needs of their patients without compromising results; these programs are listed in table 4. [For details of administration, *see* (*4*).] The regimens will not be described in detail here.

THE GOLDIE-COLDMAN HYPOTHESIS AND ATTEMPTS TO CIRCUMVENT DRUG RESISTANCE

In 1979, Goldie and Coldman (*7, 8*) applied the somatic mutation model of Luria and Delbruck (*9*) to cancer cells. They (*7, 8*) hypothesized that cancer cells develop resistance to drugs spontaneously, just as bacteria develop resistance to viruses. In their model, the resistant cell populations increased in size through normal growth and by the addition of subsequent resistant cell lines that evolved from sensitive cells. Therefore, even at normal mutation frequencies ($\pm 10^{-6}$), the likelihood of cells developing resistance was dependent on population size. The likelihood of having at least one resistant cell line present was presumed to be high at a total body burden of less than 10^9 cells, or below the level of detectability by our current diagnostic tests. This was a chilling observation and suggested to Goldie and Coldman that the major reason for drug treatment failure in the clinical setting was the development of specific and permanent drug resistance in this fashion. Their hypothesis was also the best explanation for the invariable inverse relationship between cell number and curability, so consistently demonstrated in animal models, and the effectiveness of combination chemotherapy in curing some types of human malignant neoplasms. In their models, the resistant cell population would, however, be in the minority and their presence would, theoretically, affect only the cure rate, not the response rate. An exception to this would be tumors whose growth is associated with significant cell loss. In such cancers, as many as 1,200 doublings can occur before a mass reaches 1 cm^3 in size, giving the opportunity for resistant cells to populate most of the tumor.

Table 4.—Standard chemotherapy regimens for Hodgkin's disease

MOPP
MVPP
ChlVPP[a]
ABVD
MOPP/ABVD
MOPP/ABV

[a]ChlVPP = chlorambucil-vinblastine-procarbazine-prednisone.

To overcome resistance, Goldie and Coldman suggested that all available effective drugs would need to be given early and together. Some of the clinical trials (during which patients were given alternating cycles of non-cross-resistant drugs) in Hodgkin's disease were designed intuitively and were already under way when their hypothesis was described. Initial reports suggested these clinical trials were more effective than standard programs (*10*). This led Goldie and Coldman to cite the treatment of Hodgkin's disease as an example of their hypothesis at work. Ultimately, however, this has not proven to be true, and it is now apparent that several points in their hypothesis have been misunderstood. First, their model indicated that the best approach to treatment would be the use of all effective drugs simultaneously in their *full effective doses and schedule*. Because most clinicians believe this approach is not feasible for reasons of toxicity, the approach has actually never been tested. The next best approach predicted by their model is the use of alternating cycles of non-cross-resistant *and equally effective* combination chemotherapy. If the tenets of their hypothesis were to be fulfilled, the biologic characteristics of the tumor cells, such as cell kinetic parameters, should be similar both among patients and among metastatic sites. Finally, the model is designed for researchers always to assume that the dose and schedule of the treatment programs administered in alternating cycles would be maintained at optimal levels.

A reanalysis of the Goldie-Coldman hypothesis by Day (*11*) has provided some useful insight on the difficulty of testing a hypothesis based on these assumptions because of the biologic heterogeneity of tumors, between sites and patients, and the flawed assumption that the regimens have equal efficacy. In the decade of study since the introduction of the Goldie-Coldman hypothesis, only two trials have been completed that have satisfactorily tested alternating cyclical combination chemotherapy under the assumptions originally proposed by Goldie and Coldman. The two studies will be discussed below.

A REEMPHASIS OF THE ADEQUACY OF DOSING

The most recent new data have been on the importance of the concept of dose intensity on outcome. The very first precepts of cancer chemotherapy were built around the dose–response curve. However, when dosing is involved, cancer drugs differ from drugs used to treat chronic diseases. Rather than interacting with fixed receptors to interfere continuously with metabolic functions of cells, cancer drugs attack a continuously moving target with the goal of eradicating the last cell. Because normal

dividing tissues are readily affected by their cytotoxic effects, the dose–response curve for cancer drugs is also steeper. Yet there is an extraordinary selectivity in killing of malignant cells over normal cells, as demonstrated by successful treatment of diseases, such as acute lymphocytic leukemia of childhood, Hodgkin's disease, and diffuse histiocytic lymphoma. Here large numbers of malignant cells are destroyed when patients are cured, whereas bone marrow and gastrointestinal crypt cells, although injured, always recover following the use of standard regimens.

It is striking that dosage, although an important variable, has rarely been prospectively controlled in clinical trials of the treatment of Hodgkin's disease. Only investigators conducting large studies from Stanford and the NCI have even analyzed the effect of dosage on outcome. Carde et al. (*12*) noted that as single variables, the total dose and the rate of delivery of the three cytotoxic drugs in the MOPP program had a significant effect on the ability to attain a complete remission. The mean three-cycle rate of delivery of the three cytotoxic drugs in MOPP also had significant impact on the complete response rate, especially in symptomatic patients, which suggested that the integrity of the combination was also an important factor. Finally, patients who received greater than 65% of the projected dose of mechlorethamine had a significantly better survival than those who received less than 65% of the projected dose. In a similar analysis at the NCI, Longo et al. (*13*) studied the effect of dosing on outcome of patients treated with MOPP after 20 years of follow-up. Few patients received less than 65% of the projected dose of mechlorethamine; thus the dose of this drug was not an obvious prognostic factor. The rate of delivery of vincristine over six cycles had the most significant impact on survival as a single variable and, in a regression analysis, on the complete response rate. The rate of delivery of vincristine also significantly affected the survival rate. Remission duration was affected only by the time taken to attain complete remission; those achieving remission in less than 5 months of therapy fared significantly better than those whose remission took longer than that. This may also be a dose effect because speed of response frequently reflects the impact of dosage and schedule.

At Stanford and other institutions, the dose of vincristine is capped at a total of 2 mg, regardless of body surface area. The unfortunate and nonpharmacologic practice of capping the dose of vincristine emanated from the report from Stanford by Moore and associates (*14*) because of their impression that neurotoxicity, considered moderate at the NCI, was intolerable. When the dose of vincristine is capped at 2 mg, only persons with a body surface area of 1.43 m^2 or less receive a full dose of the drug. Whereas neuropathy is common when full doses of vincristine are used, neurotoxicity often plateaus at levels associated with paresthesias, hypesthesias, weakness, and loss of deep tendon reflexes, none of which is catastrophic and all of which are reversible. At the NCI, vincristine is given in full doses unless the patient is unable to walk on heels or toes, or footdrop occurs.

When drug combinations are developed, it is rare for dosage to be considered beyond the automatic exercise of reducing doses to allow drugs to be combined, based on the physician's perception of the amount of toxicity likely to occur. This is usually performed without testing the effect of such reductions on treatment outcome. The net effect is that for most drug combination programs, when the interval between treatment cycles is considered, individual drug doses are approximately one-half the standard (single agent) dose. Compounding this dose reduction is a tendency for physicians to reduce doses randomly for a variety of nonscientific reasons, some of which are shown in table 5.

Table 5.—Commonly used methods for reduction of doses and dose rate

Round off body surface area to nearest lower tenth of a square meter.
Limit maximum dose, regardless of height and weight.
Use a fixed dose, regardless of height and weight.
Require normal blood counts for dosing.
Increase intervals between doses.
Round off vials and/or tablets to nearest unit at lower dose.
Omit a drug permanently from a combination as soon as significant toxic reactions are noted.

It was not until 1983, when Hryniuk (*15*) and Hryniuk and Bush (*16*) introduced the concept of dose intensity, that a true comparison of dosing between studies would be made. They proposed that regardless of the schedule used, dosages of all drugs in a combination be converted to milligrams per square meter per week to provide both a standard of reference between studies and a way of comparing the impact of treatment-free intervals. When comparing the dose intensity of the same combination between studies, one must use the combination with the highest doses as a standard and compare it with others by using the average of the dose intensity of the cytotoxic drugs to calculate the RDI of the program under consideration. When combinations are used that contain different drugs, drugs from a similar class can be converted to "equivalents" of a standard drug, and then the RDI is calculated and compared with the standard. If a drug that is not found in the reference standard is used, the dose intensity of that drug is counted as a zero in the calculation of the average RDI. In some circumstances, hypothetical drug combinations can be constructed with all effective drugs given in full (single agent) doses and used as a reference standard (*17*). In the past, analysis of dose has been on the basis of a comparison of percent projected protocol doses delivered. Such analyses are flawed; one cannot compare the percentage of the projected doses delivered of a drug like vincristine, when in one study the dose is given on the basis of actual body surface area and in another it is capped at a total dose of 2 mg, regardless of body surface area. Although calculation of actual dose intensity from most reported trials has not been possible, comparison of intended dose intensity, although less useful, has been accomplished. It is rare that actual dose matches intended dose; therefore, this approach can compare the dose intensities of various protocols only at the time of their design. In all studies described below, end points have also been reassembled from the original reports to isolate continuous disease-free survival at the interval between 3 and 5 years from the end of treatment, as the common end point.

When one analyzes clinical trials of new treatment of Hodgkin's disease, special attention should be given to four important variables that affect outcome. Dose intensity is the only treatment variable. The other three variables include the fraction of asymptomatic patients in each study, the fraction of patients in each study who relapse after initial treatment with radiotherapy for localized disease, and stage, with particular attention to clinical versus pathologic staging.

Table 6.—Advanced Hodgkin's disease: Clinical trials of note

MOPP repeated
MOPP vs. mechlorethamine
MOPP vs. five drugs vs. five drugs in sequence
MOPP modifications (MVPP; ChlVPP; BCVPP)[a]
Maintenance MOPP
MOPP vs. MOP
MOPP reinduction
Combination chemotherapy for stage III-A

[a]BCVPP = bleomycin-cyclophosphamide-vinblastine-procarbazine-prednisone.

Table 7.—Advanced Hodgkin's disease: Clinical trials of note

MOPP vs. BCVPP
MOPP vs. MOPP-bleomycin vs. MOP-BAP[a]
MOPP vs. ABVD
MOPP vs. MOPP/ABVD
MOPP vs. MOPP/CABS
MOPP vs. ABVD vs. MOPP/ABVD
MOPP/ABV (hybrid) vs. MOPP/ABVD
MA/MA (hybrid) vs. MOPP/ABVD

[a]BAP = bleomycin-doxorubicin-prednisone.

The absence of symptoms profoundly affects outcome in virtually all studies and is indicative either of a lower volume of tumor or a better host condition. In most studies, patients who relapse with advanced disease after radiotherapy treatment for localized disease have a significantly better complete response rate, response duration, and survival than do patients who present de novo with equivalent stages (*4*). In many studies, staging laparotomy was introduced at some time in the middle of a course of the study. Therefore, both clinical and pathologic stages should be reported for each patient group. They rarely are. Yesterday's clinical stage II-A is today's pathologic stage III-A. The differences influence outcome in ways that are not related to the treatment.

AN ANALYSIS OF TWO DECADES OF CLINICAL TRIALS

The array of clinical trials during which investigators attempted to confirm, improve, or compare the MOPP program to other programs is shown in tables 6 and 7. Detailed analyses have been provided by other investigators (*4, 18–20*).

When MOPP was first confirmed and then compared to the single agent mechlorethamine, it proved superior (*21*). In a Cancer and Acute Leukemia Group B study, MOPP was compared with a new five-drug combination and to each of the five drugs in the latter combination used in a fixed sequence (*22*). This was an important study because it was the first to show that the complete response rate alone did not predict for the quality of the remission. This regimen, which had a complete remission rate equivalent to the five-drug combination, proved superior in response duration and survival to the five-drug combination, and both combinations proved superior to the same single agents used in standard doses in fixed sequence. Modifications of the MOPP program, in which vinblastine was substituted for vincristine and chlorambucil for mechlorethamine, were found to be roughly equivalent, less neurotoxic, and associated with less nausea and vomiting.

Maintenance treatment was used in numerous studies after complete remission rate was attained with MOPP. None showed improvement in response duration and survival. This was not surprising, because patients cured with six cycles of chemotherapy were unlikely to benefit from further treatment, and those who failed to be cured should not benefit from reduced doses of the same or other combination programs.

The re-treatment of patients who relapsed after MOPP proved interesting in that those who relapsed after short remissions rarely had long second remissions, and those who had long first remissions had durable second complete remissions. These were the first data to suggest that a significant fraction of patients who relapsed after primary treatment did so because they were underdosed with the original combination (*23*), a fact that has been corroborated in recent studies on high-dose chemotherapy with autologous bone marrow transplantation support.

The use of MOPP produced a significant improvement in patients with stage III-A disease, a subset of patients thought to have a better prognosis by virtue of smaller volume of tumor or less aggressive disease. All 23 patients with either III-A or IV-A disease in the original MOPP study at the NCI attained complete remission, and only 2 have relapsed over 20 years (*1*). Subsequent studies with radiotherapy alone for asymptomatic patients have rarely reported relapse-free survival that exceeds 50% (*24*). As a result, it became standard practice for physicians to use combination chemotherapy and radiotherapy in patients with stage III-A disease to attain the same complete remission rates, relapse-free survival, and survival attained with MOPP alone. Subsequently, two randomized studies of comparisons of MVPP (a modification of MOPP in which vinblastine was used instead of vincristine) with MVPP plus radiotherapy or radiotherapy alone demonstrated superior results for MVPP versus radiotherapy with equivalent results with MVPP, compared with MVPP plus radiotherapy (*25, 26*). As a result, it is now apparent that combined modality programs with radiotherapy plus combination chemotherapy provide only toxic reactions and little benefit for most patients with stage III-A disease. Except for patients in the III-A_1 subset who do equally well with radiotherapy alone, one of the standard combination chemotherapy programs is the treatment of choice for those with stage III-A disease.

Two cooperative groups (*27*) have conducted large-scale trials that encumbered their patient populations for almost a decade while they compared MOPP to MOPP modifications and substituted or added nitrosoureas for mechlorethamine (BVCPP), or deleted a dose of mechlorethamine and added a dose of doxorubicin and bleomycin. Although the published reports of these studies contended the results were better, further analysis indicated they are no better than those attained with MOPP in the original group trials, a lack of progress not accounted for by a changing patient population. It is significant that in each of these studies, the impact of dose intensity has never been analyzed, even though dose was often compromised by random reductions and altered intervals between cycles.

ALTERNATING CYCLICAL CHEMOTHERAPY: A TEST OF THE GOLDIE-COLDMAN HYPOTHESIS

The rationale behind the design of MOPP/ABVD and MOPP/CABS was simple. About 40% of the patients with advanced Hodgkin's disease treated with MOPP and other programs were not cured by their initial treatment, either failing initial induction or relapsing after attaining a complete remission. Bonadonna et al. (*10*), Diggs et al. (*28*), and Wiernik and Schiffer (*29*) set about to develop new combination chemotherapy programs that were not cross-resistant to the MOPP program. Regimens ABVD and CABS represent two programs that contain different drugs with different toxicities. Both proved as effective as MOPP in previously untreated patients and could salvage some MOPP failures (*30, 31*). To capitalize on their use as primary therapy, physicians had essentially two choices: Treat patients with a full course (six cycles) of one combination, and then follow with a full course (six cycles) of the second combination. However, this approach would not salvage those patients who were primary MOPP failures and relapsed quickly. The other alternative they had was to use alternating cycles of each combination in a sequence. In two trials, alternating cycles of MOPP and ABVD or MOPP and CABS have been compared with remission induction with MOPP alone. There was, however, a major difference in treatment duration in these two trials. To preserve total dose, Bonadonna et al. (*10*) gave the MOPP/ABVD program for a total of 1 year or a full six cycles of each combination. In the control arm, MOPP was also given for the extraordinarily difficult duration of 12 months. In the MOPP/CABS study, the control group was given MOPP in the standard fashion, i.e., six cycles over 6 months. Alternating cycles of the MOPP/CABS combination also were given over 6 months. Only three cycles of each regimen were given in the 6-month period. Thus although dose intensity was maintained, the cumulative total dose of each drug in the alternating cyclical combination arm was less than if each was used alone.

Eighty-eight patients were randomized between MOPP or MOPP/ABVD. Seventy-four percent of the patients attained a complete remission with MOPP and 89% with MOPP/ABVD. The difference was not statistically significant. A significant difference, however, was noted in relapse-free survival favoring MOPP/ABVD, but the results are flawed by dosage problems. The results in patients with asymptomatic disease are interesting as markers of effectiveness. First, this favorable group made up 30% of the patients compared with 12% in the NCI study. Second, the results with MOPP are the worst ever reported. This appears to be related to dose reductions that were required in the MOPP program, particularly for vincristine, due to the administration over a 12-month period. Thirty-five percent of the patients treated with MOPP alone had a 50% reduction in the dose of vincristine, compared with 7% of the patients treated with MOPP/ABVD. In 9% of the patients treated with MOPP, the drug was discontinued entirely. Given the significant impact of the rate of delivery of vincristine at the NCI on remission and survival, this attentuation of the dose rate of vincristine could partly explain the poor outcome. The results of the MOPP/CABS study have not been published in detail. One hundred fifteen patients were randomized to MOPP and MOPP/CABS (*31*). Patients with massive mediastinal involvement were not included in the study because they were treated with combined chemotherapy and radiotherapy. There is no significant difference between MOPP and MOPP/CABS in complete response rates and relapse-free or overall survival.

Results of the other studies shown in table 7 will not provide data on the important question of the benefits of alternating cyclical combination chemotherapy over standard four-drug regimens. Klimo and Connors, who developed the MOPP/ABV hybrid program, used a half-cycle of each of the two combinations on days 1 and 8, respectively (*32, 33*). Their ABV is similar to ABVD except the dacarbazine is deleted. In the initial pilot trial, a complete response rate in excess of 90% was reported with a low relapse rate. In that study, however, 40% of the patients are in the favorable asymptomatic group, and the high complete remission rate was attained by boosting 15% of the population with radiotherapy to eradicate residual disease. Their MOPP/ABV regimen is now being compared with MOPP/ABVD (given in the manner developed in Italy) in a national clinical trial in Canada. This trial will not provide answers regarding the relative efficacy of alternating cyclical combination chemotherapy, because neither of the two programs is being compared with a standard four-drug program. A similar hybrid program, referred to as MA/MA, in which half-cycles of MOPP and ABVD are used on days 1 and 8, is being compared with standard MOPP/ABVD in Italy. These trials seem based on the a priori assumption that alternating cyclical combination chemotherapy represents a significant advance.

IMPACT OF DOSE INTENSITY ON OUTCOME

In view of the failure of alternating cyclical combination chemotherapy to provide an incremental benefit in Hodgkin's disease over standard four-drug combinations, it is likely that the major reason for treatment failure is underdosing during remission induction. Two lines of evidence support this hypothesis. The first comes from the re-treatment of relapsing patients. Those with remissions lasting longer than 1 year respond to re-treatment, and those who relapse in less than 1 year usually do not have durable second remissions with standard treatments. These data suggest that in the former group residual cells were still sensitive to chemotherapy, and these cells remain so, even after the tumor mass repopulates to clinically detectable levels. The other line of evidence comes from autologous bone marrow transplant programs. Patients who are highly resistant to one or more standard drug combination programs are treated with high-dose chemotherapy, often a single alkylating agent, given at three to ten times the normal dose, with autologous bone marrow transplantation support. Durable complete remissions, even with a single treatment course, are induced in approximately 50% of such patients, results not achievable at standard doses. These results suggest that the malignant cell has residual sensitivity and that the use of higher doses at the outset had obviated the need for myeloablative doses later.

Using the method of Hryniuk and Bush (*16*) to calculate dose intensity, we calculated either actual or intended dose intensity of published programs of MOPP use, including the MOPP arms of controlled trials comparing this combination with alternating cyclical combination chemotherapy (table 8). We could obtain data on actual dose intensity in only five trials. Whereas no correlation was noted between dose intensity and complete remission rate, a strong correlation was found between dose intensity and survival free of disease at 5 years (fig. 1). Of

Table 8.—Hodgkin's disease: MOPP regimens; dose intensity and outcome[a]

Study/author	RDI vs. NCI MOPP	Actual RDI	Complete response rate, %	Patients free of disease	
				Percent	At No. of yr
NCI/DeVita	1	0.85	84	55	15
Stanford/Carde	0.95	0.64[b]	72	30[c]	5
BNLI/Goldman	0.82	—	52	30	5
SEG/Huguley	0.82	0.64	46	16	2
CALGB/Nissen	0.81	—	74	37	5
ECOG/Bakemeier	0.77	0.60	73	37	5
Milan/Bonadonna	0.76	0.53-0.66	74	36	8
SWOG/Frei	0.70	—	78	31	5

[a]BNLI = British National Lymphoma Investigation; SEG = Southeastern (Oncology) Group; CALGB = Cancer and Acute Leukemia Group B; ECOG = Eastern Cooperative Oncology Group; SWOG = Southwestern Oncology Group.
[b]Actual RDI is from a prior paper.
[c]Estimate is made from patients with marrow stage IV.

particular note is the calculated actual dose intensity of the MOPP arm of the Milan trial that is between 0.53 and 0.66 of that in the NCI version of MOPP. When long-term survival free of disease after the NCI regimen is compared with the control arm of the Milan study, it appears that a reduction of dose intensity of MOPP of 38% in Milan resulted in a decrease in overall survival free of recurrence of approximately 35%.

Because of the continued debate over these issues, the Cancer and Acute Leukemia Group B is conducting a randomized trial comparing MOPP with ABVD and MOPP/ABVD. The results have not been published in detail but have been presented (*34*).

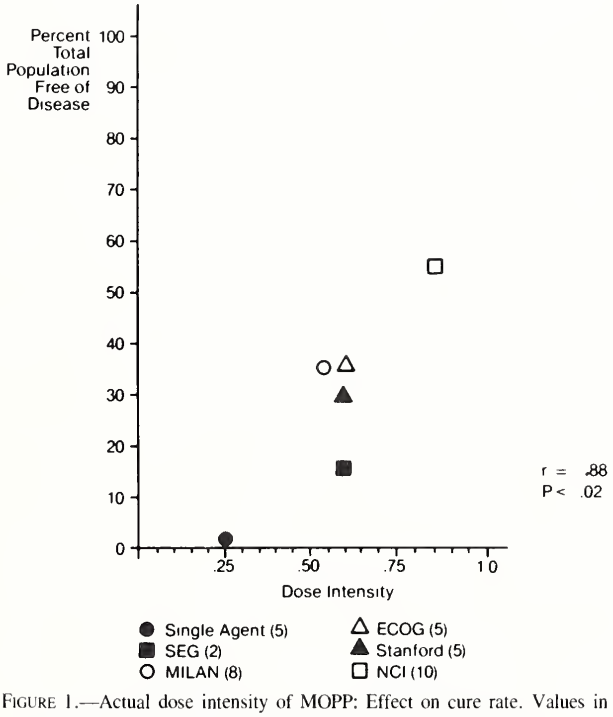

FIGURE 1.—Actual dose intensity of MOPP: Effect on cure rate. Values in parentheses represent the years at which patients are free of disease.

Preliminary evaluation indicates that ABVD is equivalent to MOPP/ABVD and both are superior to MOPP in relapse-free survival. Once again, dose intensity appears to be a factor, but it has not been analyzed by the method of Hryniuk. However, even when the percentages of projected doses of the cytotoxic drugs for all these programs were analyzed for the first three cycles of treatment, the percentages of projected doses of vincristine, mechlorethamine, and procarbazine received in the MOPP arm had been markedly reduced to 70%, 44%, and 39%, respectively, because of toxicity when compared with the dosages in the other two arms. This again appears to reflect a group bias over the side effects of the MOPP program and says that MOPP/ABVD and ABVD are superior to MOPP given in inadequate doses. The most important point thus far, however, is that ABVD in full dosage appears as good as the alternating cyclic program of MOPP/ABVD, which suggests once again that alternating cyclical combination chemotherapy is not superior to four-drug combinations used in full doses.

Figure 2 depicts a theoretical dose–response curve for Hodgkin's disease. Current data on relative dose intensity suggest that we are approximately at point B for standard combinations, at less than one-half the potential dose intensity attainable if these drugs are given in their usual schedules and at their full individual doses. Ablative chemotherapy supported by autologous bone marrow transplantation has defined the upper end of the dose–response curve (fig. 2, point D). We are not at

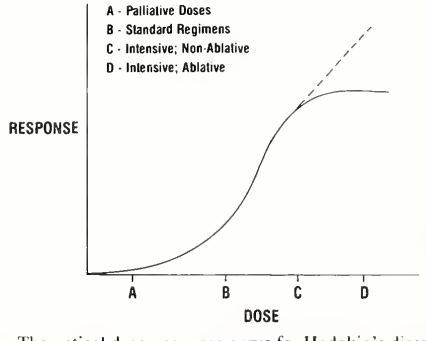

FIGURE 2.—Theoretical dose–response curve for Hodgkin's disease.

Table 9.—Clues to maximizing dose intensity in Hodgkin's disease

Clinical observation	Interpretation
Patients who relapse after a long remission respond well to the same regimen.	They were underdosed during primary treatment and were not resistant to chemotherapy.
Reduction of dosages of alkylating agents in MOPP adversely influences outcome.	Dosages of alkylating agents are important; agents are essential components of treatment.
Patients who fail to benefit from MOPP respond to a single alkylating agent as salvage.	Patients were underdosed with alkylating agents and are only relatively resistant.
Alkylating agents at 3–10 times normal doses are a major component of successful autologous bone marrow transplantation programs in patients "resistant" to all standard regimens containing alkylating agents.	Resistance of alkylating agents is relative, and dosage is very important.
Patients who fail to benefit from MOPP respond to single use of procarbazine as salvage treatment.	Procarbazine dose in MOPP is too low.
Vincristine rate of delivery is an important treatment variable.	Body surface area is used as basis. Neurotoxicity limits further escalation.

all certain, however, whether ablative doses of chemotherapy are required, and investigators are currently exploring the upper reaches of the dose–response curve earlier in the disease by using more dose-intense versions of standardized combinations to control the dose variable. We provide some clues to improve dose intensity of such standard programs in table 9. Taking these into consideration, we modified the standard MOPP program to increase the dose intensity of all the major drugs, as shown in table 10, in a pilot trial and support this increase with the use of granulocyte/macrophage colony-stimulating factor. Our goal is to test the use of intense, but non-marrow-ablative, chemotherapy as primary treatment as an alternative to high-dose chemotherapy with autologous bone marrow transplant support.

THE INVARIABLE INVERSE RELATION BETWEEN CELL NUMBER AND CURABILITY

There is an invariable inverse relationship between cell number and curability in all animal tumor models (*35*), and clinical data suggest a similar relationship in human tumors, such as breast cancer (*36*). Drug programs that cure advanced Hodgkin's disease should then be more effective in patients with early stage disease. We also appear to have reached a limit in the curability of early stage disease by radiotherapy, and any attempts to improve results have almost exclusively been limited to combining chemotherapy with radiotherapy. This has resulted in some improvement in relapse-free survival, and in a few studies, a small improvement in survival has been observed, but at a very significant cost in long-term toxic effects (*37, 38*). As with patients with stage III-A disease, in whom combination chemotherapy appears as good as or better than combined use of radiotherapy or combination chemotherapy, we should be able to provide the incremental improvement over current radiotherapy results by using chemotherapy alone if the inverse rule holds. We have tested this hypothesis by randomly assigning patients with stage II disease, excluding those with massive mediastinal involvement, to either full-dose MOPP or radiotherapy (*39*). All patients were staged by laparotomy to determine precisely the sites of tumor involvement. The complete response rate in early stage disease is 100% with MOPP, and, thus far, MOPP appears at least equal and perhaps superior to radiotherapy in relapse-free and overall survival. A similar study has been conducted by Cimino et al. (*40*). Results of this latter study indicate that MOPP and radiotherapy produce equivalent results, except that patients who relapsed after MOPP have a higher probability of dying than do those who relapse after radiotherapy, although the number of patients who have relapsed is small, and this difference has not reached statistical significance. In-field recurrences were more common in the MOPP group. The authors provided insufficient data to

Table 10.—Comparison of dose intensity of MOPP vs. DIMOPP[a]

Agent	MOPP, $mg \cdot m^3 \cdot wk^{-1}$	DIMOPP, $mg \cdot m^3 \cdot wk^{-1}$	Increase, %
Mechlorethamine	3	4.8	+60
Vincristine	0.7	0.93	+33
Procarbazine	350	467	+33
Prednisone	140	187	+33

[a]DIMOPP = dose-intense MOPP.

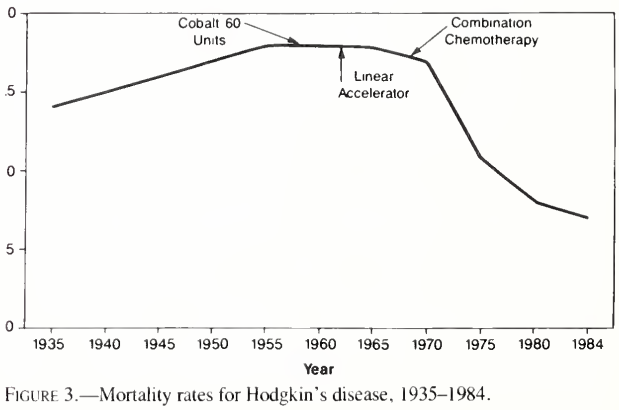

FIGURE 3.—Mortality rates for Hodgkin's disease, 1935–1984.

calculate dose intensity in the MOPP program used. Other studies have been reported supporting the thesis that combination chemotherapy is effective in early stage disease (*41–44*), although some are flawed by ad hoc modifications in the drug doses (*44*). Thus the data support the applicability of the inverse rule in Hodgkin's disease. Long-term follow-up of patients in these studies will be required if we are to determine the impact of the different side effects of treatment on the ultimate outcome. Finally, figure 3 illustrates the significant impact that treatments developed in the past two decades for Hodgkin's disease have had on national mortality. Mortality rates for Hodgkin's disease began to level off with the introduction of the cobalt-60 units and linear accelerators and declined rapidly with the introduction of combination chemotherapy (*4*).

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Treatment of Patients With Aggressive Lymphomas: An Overview[1]

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ABSTRACT—The treatment of aggressive lymphomas has progressed to the point that over 90% of the patients with localized disease, around 50% of the patients with advanced disease, and perhaps up to 25% of relapsing patients may enjoy long-term, disease-free survival. Several studies have documented that primary combination chemotherapy with or without involved-field radiation therapy is capable of curing 90% or more of the patients with clinically staged, localized disease. Results with primary radiation therapy in such patients are not nearly as good, even in those who undergo laparotomy staging. The management of advanced-stage disease is more controversial. Although combination chemotherapy is the treatment of choice, there is debate about whether the impressive results with the latest regimens, such as MACOP-B, COP-BLAM III, and ProMACE-CytaBOM, which appear to be curative for around 60% or more of advanced-stage patients, are really superior to CHOP, the most widely used program, which cures about 30% or so of the patients. A major idea that is guiding the development of new treatment programs is augmenting the dose intensity of the treatment. This notion is fueled not only by the demonstrated dose–response relationships that have been documented by Hryniuk and colleagues in other tumor types, such as cancers of the breast, ovary, and colon, but also by recent experience in the salvage therapy of relapsed aggressive lymphoma. Formerly a universally fatal disease, relapsed aggressive lymphoma now appears to be responsive to high-dose chemotherapy with or without radiation therapy followed by autologous or allogeneic bone marrow or peripheral blood stem cell reconstitution. The capacity of high-dose chemotherapy to cure a fraction of these patients, who are resistant to conventional-dose chemotherapy, suggests that primary therapy is further improved by strategies aimed at delivering therapy at a higher dose intensity. The development of colony-stimulating factors and new insights in the biology of hematopoiesis make it possible to imagine many innovative approaches to this question.—J Natl Cancer Inst Monogr 10:29–37, 1990.

The malignant lymphomas are a morphologically, immunologically, and genetically diverse group of malignant neoplasms. Our current capacity to cure a large fraction of patients with certain malignant lymphomas is a result of the development of 1) a systematic approach to staging the extent of disease, 2) large-field radiation therapy techniques, 3) aggressive combination chemotherapy treatment programs, and, perhaps most important, the development by Henry Rappaport of a simple, fairly reproducible classification scheme (*1*). This scheme, with minor modifications, has turned into the Working Formulation (*2*) and is still the most useful schema more than 35 years following its original description. Rappaport grouped the lymphomas based on their pattern of growth [nodular (now follicular) vs. diffuse] and the morphology of the predominant malignant cell [well-differentiated (now small lymphocytic), poorly differentiated (now small cleaved cell), histiocytic (now large cell), or mixed] and came up with an easily learned and reproducible lymphoma classification. The Rappaport classification provides the clinician with the best single predictor of the behavior of the diseases. This is even more remarkable considering that the Rappaport scheme predated the development of reliable clinical data on the natural history of the diseases. However, the natural history was accurately predicted by the Rappaport diagnosis (*3*). Minor additions and alterations in the Rappaport scheme have been codified as the Working Formulation, and it, too, requires some modifications to be truly valuable to the clinician.

Currently, the non-Hodgkin's lymphomas are divided into three groups based on their clinical behavior: 1) indolent or low grade, 2) aggressive or intermediate grade, and 3) high grade (table 1). The treatment of choice for indolent lymphomas is the subject of ongoing investigation. Radiation therapy is probably curative for the vast majority of patients with truly localized disease; however, only about 15% of patients have localized disease. In advanced-stage patients, no initial therapy followed by palliative treatment with local radiation therapy, single-agent

ABBREVIATIONS: NCI = National Cancer Institute; CHOP = cyclophosphamide–doxorubicin–vincristine–prednisone; ProMACE-MOPP = prednisone–methotrexate–doxorubicin–cyclophosphamide–etoposide–vincristine–mechlorethamine–procarbazine; MOPP = mechlorethamine–vincristine–procarbazine–prednisone; C-MOPP = cyclophosphamide–vincristine–procarbazine–prednisone; BACOP = bleomycin–doxorubicin–cyclophosphamide–vincristine–prednisone; COMLA = cyclophosphamide–vincristine–methotrexate–leucovorin–cytarabine; ACOMLA = doxorubicin plus COMLA; M-BACOD = high-dose methotrexate–bleomycin–doxorubicin–cyclophosphamide–vincristine–dexamethasone; COP-BLAM = cyclophosphamide–vincristine–prednisone–bleomycin–doxorubicin–procarbazine; MACOP-B = methotrexate–doxorubicin–cyclophosphamide–vincristine–prednisone–bleomycin; CytaBOM = cytarabine–bleomycin–vincristine–methotrexate; F-MACHOP = fluorouracil–methotrexate–cytarabine–cyclophosphamide–doxorubicin–vincristine–prednisone; MIME = mitoguazone–ifosfamide–methotrexate–etoposide; HOAP-bleo = doxorubicin–vincristine–cytarabine–prednisone–bleomycin; OAP-bleo = HOAP-bleo without doxorubicin.

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Table 1.—NCI Clinical Schema for malignant lymphomas

Indolent
Small lymphocytic
Follicular, small cleaved cell
Follicular, mixed
Diffuse, small cleaved cell[a]
Diffuse, intermediate[b]
Aggressive
Follicular, large cell
Diffuse, mixed
Diffuse, large cell
Diffuse, immunoblastic[c]
Highly aggressive (leukemia-like)
Diffuse, small noncleaved cell (Burkitt's and non-Burkitt's)
Lymphoblastic
Adult T-cell leukemia/lymphoma[b]

[a] This is classified as intermediate grade in the Working Formulation but considered clinically indolent based on experience at the NCI.

[b] This type is omitted from the original Working Formulation.

[c] Classified as high grade in the Working Formulation, this type is considered clinically aggressive based on experience at the NCI.

chemotherapy with or without steroids, and combination chemotherapy are all effective at producing disease regression (*4*). However, there is no evidence that the natural history of the disease is affected by treatment (*5*). The high-grade lymphomas appear to be best treated as one would treat acute lymphoblastic leukemia. These lymphomas tend to occur in children and young adults, have very rapid progression rates, and often involve the bone marrow and central nervous system. Thus therapy should consist of high-dose multidrug induction, consolidation, and maintenance programs with central nervous system prophylaxis.

The subject of this review is the treatment of the intermediate-grade or aggressive lymphomas, which, according to the NCI Clinical Schema, include follicular large cell (histiocytic), diffuse large cell (histiocytic), diffuse immunoblastic, and diffuse mixed cell lymphomas (*6*). Note that immunoblastic lymphomas are considered histologically high grade based on the Working Formulation. However, the NCI and other treatment centers have found that their clinical behavior is more analogous to the other aggressive lymphomas than to Burkitt's or lymphoblastic lymphoma.

AGGRESSIVE (INTERMEDIATE-GRADE) LYMPHOMAS

About 70% of all lymphomas are non-Hodgkin's lymphomas, and diffuse aggressive lymphomas account for about 60% of these. Most are B-cell-derived tumors; 20% are of T-cell origin, and most of these occur in the diffuse mixed lymphoma and immunoblastic categories; only a small percentage are true histiocytic tumors.

The aggressive lymphomas often present with extranodal primaries (~40%), localized disease (~25%–30%) and B symptoms (~45%). Unlike patients with indolent lymphomas, most patients (70%) with aggressive lymphoma who do obtain a complete remission are usually cured of their disease. A number of prognostic factors distinguish patients with a distinct natural history and probability of response to therapy. Initial attempts to determine the prognosis of patients with intermediate-grade lymphoma focused on extent of disease using the staging system borrowed from Hodgkin's disease. However, this staging system fits the patterns of disease of the non-Hodgkin's lymphomas poorly. The Ann Arbor staging system (*7*) is based on the predictable stepwise contiguous nodal spread of Hodgkin's disease, a natural history dissimilar to the often blood-borne dissemination that occurs in patients with non-Hodgkin's lymphomas. The intermediate-grade lymphomas, in particular, commonly originate in extranodal sites, such as the liver, bone, and gastrointestinal tract, and early in the course of disease, systemic dissemination may occur, usually by hematogenous spread. Unlike with Hodgkin's disease, most patients with non-Hodgkin's lymphoma present with advanced-stage disease (*8*). Furthermore, enormous heterogeneity is seen in the natural history of patients with the same stage of disease. Patients with stage II disease may have excellent or dismal prognoses based on individual features of the disease. The features that seem to have prognostic significance independent of stage differ a little from one treatment center to another but, in general, they are surrogate measures of tumor bulk and the patient's physiologic reserve, including poor performance status, B symptoms, lactate dehydrogenase levels over 250 or 500 U/dL, bone marrow involvement, and large (>10 cm) intra-abdominal mass (*9*). Other factors that may adversely affect response to therapy include more than three sites of tumor involvement, advanced age [but this is not universal (*10*)], and slow rate of response to initial therapy (*11*). Good performance status and the absence of these clinical features are associated with favorable outcome to treatment, and poor performance status with all or any of these clinical features is associated with a poorer treatment outcome. Because of the inadequacy of the current staging system, considerable enthusiasm has been shown for construction of a new system based on the known clinical prognostic factors (one such system is proposed in table 2). Such a system will probably have its defects, however, but as treatment becomes more effective, the need to subdivide patients into prognostic categories will diminish.

Once the diagnosis of non-Hodgkin's lymphoma is made, a staging evaluation is performed. Staging should include physical examination, complete blood count, serum chemistries, electrocardiogram, chest x ray, bipedal lymphangiography (computerized tomography scan of the abdomen may be useful as an adjunct but should not be used instead of a lymphangiogram), bone scan, bilateral bone marrow aspirates and biopsies,

Table 2.—NCI Modified Staging Schema for aggressive lymphomas

Stage	Description
I	One or two nodal sites or one extranodal site of disease without poor prognostic features
II	More than two nodal sites of disease or one or more localized extranodal sites plus draining nodes with none of the following poor prognostic features: performance status ≤70, B symptoms, any mass >10 cm in diameter (particularly abdominal), serum lactate dehydrogenase > 500 U/dL, bone marrow involvement, three or more extranodal sites of disease
III	Stage I or II plus any poor prognostic feature

and evaluation of the liver, preferably with multiple percutaneous biopsies performed under direct visualization at peritoneoscopy. Patients with positive bone marrows should undergo lumbar puncture because they are at an increased risk of developing lymphomatous meningitis (*12*). Upper and lower gastrointestinal series and intravenous pyelography should be done only if symptoms or signs point to gastrointestinal tract or renal involvement. All suspected sites of extranodal involvement require pathologic verification. The least morbid, but usually reliable, technique is often fine needle aspiration under fluoroscopic or ultrasonic guidance. There is evidence that resection of large stomach or other gastrointestinal primary lesions may improve the subsequent response to therapy and favorably affect long-term survival, but this approach has not been evaluated in a prospective randomized study (*13*). Unlike Hodgkin's disease, exploratory laparotomy is not a routine part of lymphoma staging and is reserved for those patients presenting with undiagnosed symptoms or mass lesions in the peritoneal cavity. At the end of therapy, tests that were positive at initial staging are repeated.

TREATMENT OF AGGRESSIVE NON-HODGKIN'S LYMPHOMA

Localized Disease

Approximately 40% of the patients with aggressive non-Hodgkin's lymphoma will have localized disease. Radiation therapy was the first treatment approach to produce long-term, disease-free survival in patients with localized aggressive lymphoma (*14*). The efficacy of involved-field radiation therapy in the treatment of patients with stages I, I-E, II, and II-E aggressive lymphoma depends on three major variables: the extent of staging (laparotomy done or not), the extent of disease (large mass, high lactate dehydrogenase levels, B symptoms or not, number of involved sites), and the extent of radiation therapy (involved field, extended field, or total nodal). Studies in which patients are clinically staged (no laparotomy) report 5-year survival rates of 65% for stages I and I-E patients but only 25% for stages II and II-E patients (*15*). On the other hand, stage I patients whose staging includes laparotomy may have long-term survival rates approaching 100% (*16*). Thus it would appear that radiation therapy alone is most effective in patients who have small volume stage I disease and whose staging has included exploratory laparotomy (*17, 18*).

One reason for the failure of radiation therapy to cure a larger fraction of patients is the propensity of aggressive lymphomas to spread systemically early in the course of disease. For this reason, a number of investigators have added combination chemotherapy to irradiation for the treatment of localized aggressive lymphoma. Several who conducted prospective randomized studies (*19–22*) suggest that the addition of combination chemotherapy [even drug regimens that are not effective in advanced disease, e.g., cyclophosphamide, vincristine, and prednisone (CVP)] to radiation therapy significantly improves the treatment outcome (table 3). A number of investigators have tested a more effective chemotherapy regimen (CHOP) in combination with radiation therapy for clinically staged stage I and II patients (*23–25*). Long-term disease-free survival was 80%, a level of success at least as high as the best results in patients treated with radiation. Similarly, we (*24*) have obtained excellent results in stage I and I-E patients treated with a modification of ProMACE-MOPP (75% doses of myelosuppressive drugs and four treatment cycles rather than six) followed by involved-field radiation therapy. Forty-five of 47 (96%) clinical stage I or I-E patients achieved a complete remission, and no patient has relapsed; median follow-up time was 42 months. Thus it appears that the use of an active combination chemotherapy program with the addition of radiotherapy as the primary treatment offers a high probability of a successful outcome without subjecting the patient to the risks associated with exploratory laparotomy. Although these regimens are for the most part well-tolerated, outpatient treatment programs with mild acute toxicity, there is the potential for long-term side effects. Acute leukemia does not seem to be a

Table 3.—Results of treatment in patients with localized aggressive lymphoma after radiation therapy, chemotherapy, or both

No. of patients	Stages	Laparotomy	Treatment[a]		5-yr disease-free survival, %	Reference
			Radiation	Chemotherapy		
17	I	Yes	X	—	94	(*16*)
14	II	Yes	X	—	56	
9	I	Yes	EF	—	100	(*21*)
27	I	No	X	—	56	(*15*)
64	II	No	X	—	25	
36	I	No	X	—	61	(*18*)
38	II	No	X	—	42	
37	I and II	No	X	—	45	(*19*)
31	I and II	No	X	CVP	76	
108	I and II	No	IF	CHOP	82	(*23*)
34	I and II	No	—	CHOP	78	
47	I	No	IF	ProMACE-MOPP[b]	96[c]	(*24*)
43	I and II	No	—	CHOP[d]	~70	(*25*)
				For stage I	91	

[a] X means delivery of large-field irradiation was not further specified. EF = extended field; IF = involved field.
[b] Doses of myelosuppressive agents were reduced by 25% from the standard regimen, and only four cycles of therapy were given.
[c] Disease-free survival was 4 yr.
[d] A number of different regimens were used, but most patients received CHOP.

problem even after combined modality treatment when short-term combination chemotherapy plus involved-field radiation therapy is administered. However, given the efficacy of chemotherapy alone in advanced-stage patients, one can reasonably ask whether combined modality therapy is necessary. The data from Jones et al. (*23*) suggest that chemotherapy alone is as effective as combined modality treatment, and Cabanillas has reported on the efficacy of chemotherapy alone in the treatment of patients with localized aggressive lymphomas (*25*). Only small numbers of patients have been treated, however, and a prospective trial examining the relative efficacy of chemotherapy alone or combined with involved-field radiation therapy has not been performed.

We recommend that patients with early stage lymphoma of aggressive histology not undergo exploratory laparotomy. Although the best treatment approach has not been demonstrated conclusively by prospective randomized trial, we would suggest that clinically staged, early stage patients (stage I in table 2), with fewer than three sites of disease and no bulky abdominal masses, receive either CHOP or modified ProMACE-MOPP combination chemotherapy for four to six cycles followed by involved-field radiation therapy. Patients with Ann Arbor stage II or II-E disease with 3 or more sites of disease or an abdominal mass greater than 10 cm (stages II and III in table 2) should be managed similarly to patients with advanced-stage disease.

Advanced Disease

Results of treatment for advanced-stage diffuse aggressive lymphomas have improved over the last 15 years (*26, 27*). Before DeVita and his colleagues introduced the use of MOPP and C-MOPP (*28*), 5-year survival with advanced-stage aggressive lymphoma was essentially zero. With the use of MOPP and C-MOPP, about 45% of the patients achieved a complete remission; with a median follow-up of more than 14 years, 37% of all patients remained free of disease. Multiple attempts have been made to improve results of these basic regimens; two regimens named BACOP (*29, 30*) and COMLA (*31*) have not appreciably augmented response rates or survival.

Based on phase II studies with single agent doxorubicin, McKelvey and his associates (*32*) developed the CHOP regimen, which gained immediate acceptance in the medical community because it had the highest complete remission rate (58%) of any regimen reported at the time, it was easy to give, and it was easy to take, with minimal nausea and only moderate toxic effect on marrow. The initial reports seemed to show, however, that 50% or more of the complete responders had relapsed within the first 2 years, and some were concerned that the durability of the complete responses would be limited. However, Armitage et al. (*33*) initially reported more reassuring long-term follow-up data that about one-third of CHOP-treated patients were cured. More recently, Coltman and his colleagues (*34*) have reviewed the experience of the Southwest Oncology Group in which more than 400 patients were treated with CHOP-based programs. The complete response rate is 53%, and 30% of all treated patients achieved durable complete remissions with no relapses after 7 years. Attempts to improve CHOP by adding bleomycin, levamisole, and bacille Calmette-Guérin have had no significant impact on treatment outcome (*35*). It is useful to think of MOPP, C-MOPP, BACOP, COMLA, and CHOP and its variants as the first-generation treatment programs. Each regimen appears capable of inducing complete remissions in about 50% of all patients, and about one-third of them with advanced-stage diffuse aggressive lymphoma appear to be cured. Most patients who relapsed from complete remission did so within the first 2 years after the end of treatment.

The second generation of aggressive lymphoma treatment programs were developed in the late 1970s. Different research groups set out in different directions, yet like the first-generation programs, the second-generation programs achieved similar rates of success, despite their dissimilar hypotheses. The Dana-Farber Cancer Institute added high-dose methotrexate to the BACOP regimen and switched from prednisone to dexamethasone for better control of central nervous system relapse; use of M-BACOD resulted in complete responses in 75% of the patients, and 55% to 60% were projected to be cured at the time of the initial report (*36*). Similar results were observed when the dose of methotrexate was reduced from 3 g/m^2 to 200 mg/m^2 (*37*).

Coleman and co-workers (*38*) at the New York Hospital also chose to build on BACOP; they shortened each cycle to 3 weeks and added 10 days of treatment with procarbazine (*38*). Their regimen, COP-BLAM, induced complete remissions in 73% of the patients, and overall survival appeared to plateau at 55%.

Investigators at the NCI used ProMACE-MOPP flexitherapy, a scheme based on the delivery of a new five-drug regimen ProMACE for a flexible number of cycles based on the rate of response (*39*). Patients whose rate of response slowed in response to ProMACE were switched on their next cycle to MOPP chemotherapy, which contained three drugs to which their tumors had not been exposed. After achieving a complete response or after the rate of response to MOPP slowed, patients were switched back to ProMACE in an effort to consolidate the complete response. Complete responses were obtained in 77% of the patients with stages III and IV disease. After a median follow-up of more than 9 years, 40% of the complete responders have relapsed, and about one-half of all the patients appear to be cured of their disease. About 25% of the relapses occurred later than 2 years after therapy, and these patients by and large recurred with indolent lymphomas.

Cabanillas and colleagues (*40*) also attempted to alter the timing of administration of agents to minimize the emergence of drug resistance. Patients were given three cycles of CHOP chemotherapy, and those not attaining complete remission were switched to HOAP-bleo, in which cytarabine and bleomycin were substituted for cyclophosphamide. Patients achieving complete remission with initial CHOP received OAP-bleo (HOAP-bleo without doxorubicin). Those who completed CHOP and HOAP-bleo received ifosfamide, methotrexate, and etoposide either as consolidation or salvage therapy. The complete response rate for advanced-stage patients was over 70%, and although data regarding durability are limited, it is projected that about 50% of advanced-stage patients may be cured by this regimen.

Two groups have tried to improve the treatment of advanced aggressive lymphoma by making alterations in the COMLA regimen. Todd and co-workers (*41*) added doxorubicin (ACOMLA) and obtained a complete response rate of 65%, and 75% of these responses were durable. Baer et al. (*42*) gave the COMLA regimen, with cyclophosphamide delivered every 6 weeks instead of the original every-12-week schedule. Such "dose-intense" COMLA obtained complete responses in 80% of

the patients and a long-term disease-free survival of around 60%. This was the first demonstration that altering dose intensity might improve treatment outcome in lymphoma.

Thus in most of the second-generation programs, the major changes involved the addition of more active drugs. Such changes resulted in complete remission rates of 75%, with the likelihood of curing approximately 50% of the patients with advanced-stage disease. These apparent improvements must be examined carefully, because these regimens generally have not been compared in prospective randomized trials and are therefore subject to the problems associated with the use of historical controls. Median follow-up varies among studies, and all have included patients less than 2 years from remission induction who must be considered at risk for relapse. Some studies included stage I and/or stage II patients, who may have a more favorable prognosis, and some have added radiation therapy to sites of bulky disease, a practice that has not been prospectively evaluated and should be discouraged until data support its use. [In fact, a recent review of patterns of relapse suggests that most patients do not relapse solely in previous sites of bulky disease (*43*)]. The studies vary in their criteria for patient selection and stratification for known clinical prognostic factors. Despite these potential drawbacks, we believe that the improved remission rates and consistent durability of remission represent real progress over the first-generation regimens.

The third-generation treatment regimens that appear capable of inducing complete remissions in more than 80% of all patients and cures in up to two-thirds of the patients have been focused on alterations in doses and schedules to augment drug intensity rather than the addition of new drugs. The COP-BLAM III regimen alters the delivery of vincristine and bleomycin by administering continuous infusions of these agents for 2 and 5 days, respectively, while alternating during every other cycle with bolus vincristine and delivering the other four agents similarly to the original COP-BLAM (*44*). Complete remissions were obtained in 86% of the patients, and the plateau in the survival curve is projected at 70% (*45*). Results with three other regimens, MACOP-B (*46*), ProMACE-CytaBOM (*47*), and F-MACHOP (*48*), suggest that the use of high doses of many drugs early in intensive short-course treatments will improve results.

The MACOP-B regimen consists of six drugs. Treatment is given for only 12 weeks, during which cyclophosphamide and doxorubicin are alternated with vincristine and methotrexate or bleomycin. Prednisone and cotrimoxazole are given orally throughout the 12 weeks of therapy. Pooled long-term results for 126 patients with intermediate-grade aggressive lymphomas showed an 86% complete response rate (*49*). Sixty-seven percent of complete responders remain in complete remission, and overall survival for all patients is 65% at 78 months of follow-up.

F-MACHOP consists of seven drugs administered sequentially within the first 3 days of a 3- or 4-week treatment cycle. Fifty-six patients were treated and 43 achieved a complete remission (77%), with 80% of the complete responders projected to be alive and disease free at 4½ years (*48*). This is true even though the authors included patients with lymphoblastic lymphoma and small noncleaved cell lymphomas, both high-grade lymphomas generally less effectively treated by regimens designed for intermediate-grade lymphomas.

ProMACE-CytaBOM consists of eight drugs: cyclophosphamide, doxorubicin, and etoposide are given on day 1; cytosine arabinoside, bleomycin, vincristine, and methotrexate are given on day 8; prednisone is given on days 1 to 14; and cotrimoxazole is given throughout the 17 weeks of therapy. Treatment is given for 2 weeks out of 3 for six cycles. We compared this regimen to a modification of ProMACE-MOPP that was described earlier. Instead of giving ProMACE and MOPP in different months, the drugs were alternated on days 1 and 8 of each cycle. Of 193 patients who could be evaluated, 99 received ProMACE-MOPP and 94 received ProMACE-CytaBOM. The complete remission rates were 74% and 86%, respectively. The relapse rate was 41% in the ProMACE-MOPP arm and 27% in patients receiving ProMACE-CytaBOM (two-sided $P = .097$). A significant incidence of *Pneumocystis carinii* pneumonia was observed in the ProMACE-CytaBOM arm, with a number of early deaths. This problem was eliminated by having all patients on the ProMACE-CytaBOM arm take cotrimoxazole throughout treatment. When deaths due to *Pneumocystis carinii* pneumonia were excluded, overall survival was significantly higher in the ProMACE-CytaBOM group (two-sided $P = .036$).

Thus MACOP-B emphasizes dose intensity, F-MACHOP early exposure to around twice as many drugs as the usual regimen, and ProMACE-CytaBOM both dose intensity and more drugs. All three programs induce complete remissions in about 80% or more of the patients, and, if current projections of relapse rates hold true with longer follow-up, from one-half to two-thirds of the patients may be cured.

Coiffer et al. (*50*) have also obtained excellent results in patients with aggressive lymphomas (as defined in table 1) using a regimen called LNH-80, which consists of three intensive courses of doxorubicin, cyclophosphamide, vindesine, bleomycin, and methylprednisolone. This is followed by consolidation with cytarabine, high-dose methotrexate, and L-asparaginase followed by intensification with cyclophosphamide, teniposide, bleomycin, cytarabine, and methylprednisolone. The regimen also includes prophylactic treatment of the central nervous system with intrathecal methotrexate. The complete response rate was 81% among the 70 patients with aggressive lymphoma. The relapse rate was 20%, and the expected 5-year disease-free survival is greater than 60%. Although the results in aggressive lymphoma are as good as those achieved with other regimens, the 8-month treatment time and intrathecal therapy are probably excessive. This regimen appears more suitable for the high-grade lymphomas.

Table 4 summarizes the response data for the regimens currently used to treat patients with advanced-stage aggressive lymphomas. Clearly, the increase in complete remission and long-term survival rates has been progressive, such that now a number of regimens are capable of inducing prolonged disease-free survival in 60% or more of the patients. These improvements must be examined carefully, because these regimens have not been compared in prospective randomized trials; therefore, their interpretation is subject to the same pitfalls discussed earlier. Although it is possible that differences in tumor burden, patient age, etc., might explain small differences between certain regimens, it is unlikely that these factors can explain the large differences between the current regimens and other earlier treatment programs like CHOP. The addition of new and more active drugs and the knowledge of the importance of dose intensity are most likely responsible for these improvements (*51*).

Table 4.—Combination chemotherapy for advanced-stage aggressive non-Hodgkin's lymphoma

Regimen	Complete remission, %	Long-term survival, %[a]	References
CHOP	53	30	(*34*)
COP-BLAM	73	55	(*45*)
M(m)-BACOD	72	48	(*36, 37*)
ProMACE/MOPP flexitherapy	77	50	(*39*), this report
ProMACE-MOPP	74	53	(*47*), this report
CHOP/HOAP-bleo/IMVP-16[b]	82	NA	(*40*)
ACOMLA	65	48	(*41*)
Dose-intense COMLA	80	60	(*42*)
COP-BLAM III	86	70	(*44*)
MACOP-B	86	65	(*46*)
F-MACHOP	77	60	(*48*)
ProMACE-CytaBOM	86	69	(*47*), this report
ACVB[b]	81	63	(*50*)

[a]Survival may be from 3 to 7 yr of follow-up. NA = not applicable.
[b]IMVP-16 = ifosfamide-methotrexate etoposide; ACVB = cyclophosphamide-vindesine-bleomycin-methylprednisolone.

The first prospective randomized comparison of a first-generation (CHOP) to a second-generation (COP-BLAM) regimen was reported by Gerhartz et al. (*52*). The complete response rates [11 of 29 (38%) for CHOP and 23 of 27 (85%) for COP-BLAM] and the overall survival significantly favor the second-generation program. Numerous other prospective randomized trials are under way to compare the second- and third-generation treatment protocols with CHOP chemotherapy. Until the results of such studies are known, it would seem most prudent for physicians to deliver one of the second- or third-generation treatment protocols to patients with advanced-stage aggressive lymphoma. The death rate due to toxic effects of such treatment programs is 5% or less, and although some doubt exists about whether all of them are superior to CHOP, there is little doubt that they are at least as good.

SALVAGE THERAPY FOR PATIENTS WITH AGGRESSIVE LYMPHOMA

Few data are available on the effects of salvage combination chemotherapy in patients with aggressive lymphoma who relapse from a radiation therapy-induced complete remission. Armitage and Wen (*53*) treated 20 such patients primarily with CHOP and found a lower complete response rate (35%) and a lower 5-year survival (20%) than for patients receiving the same therapy who had not previously relapsed. Similarly, patients with advanced-stage disease who relapse after a chemotherapy-induced remission appear to have very little chance of long-term survival with a conventional dose of salvage combination chemotherapy. Cabanillas and his colleagues have had the most success in the salvage setting. Using the MIME regimen (mitoguazone, ifosfamide, methotrexate, etoposide), he and his associates have obtained complete responses in around 27% (35 of 131) of the patients with relapsed aggressive histology lymphoma (*54*). The median remission duration is about 15 months, and 10% of the patients appear to enjoy prolonged disease-free survival with this regimen. They have also used dexamethasone, cytarabine, and cisplatin (DHAP) in a similar population of patients and obtained complete responses in 31% (22 of 72), with one-half of the complete responses lasting beyond 2 years (*55*); thus 10%–15% of the patients treated with this combination may be cured.

The use of high-dose therapy with autologous or allogeneic bone marrow transplantation appears to have improved remarkably the outlook of the patient who fails primary treatment. Reports on well over 500 patients have documented long-term survival rates between 22% and 69% (*56–62*). The induction regimen usually consists of high-dose cyclophosphamide plus fractionated total-body irradiation. Some groups have included in vitro bone marrow purging with antibody plus complement, magnetized antibody separation, immunotoxins, or chemotherapeutic agents, even though the role of purging is not clear. It appears that patients who are not in complete remission at the beginning of the preparative regimen fare more poorly than those who are disease-free after conventional-dose, salvage combination chemotherapy. The general experience is that there is no point in subjecting patients to transplantation who have disease refractory to or progressing on conventional-dose chemotherapy salvage programs. It is not yet clear that variations on the standard preparative regimen make a significant impact on outcome. Treatment-related mortality should be 5% or less in experienced hands. At this point, the major difficulty is that all of the series involve highly selected patient populations. Therefore, it is not clear what fraction of relapsed patients may benefit from the aggressive salvage approach.

In an effort to address this question, we have embarked on a study designed to determine the fraction of all relapsed patients who might be salvaged by an aggressive therapy program including bone marrow transplantation. We evaluated 51 patients who had either relapsed from a complete remission or were induction failures after therapy with ProMACE-MOPP or ProMACE-CytaBOM (table 5). Nine (18%) either refused to undergo aggressive salvage therapy (5) or were medically unable to receive such treatment (4). The remaining 42 (82%) were treated with either ProMACE as delivered in the flexitherapy regimen (*39*), if they had relapsed from complete remission, or high-dose cisplatin plus etoposide, if they had been induction failures. Those failing to obtain a complete remission or more than 90% partial remission in response to ProMACE were offered treatment with cisplatin plus etoposide, and those failing

Table 5.—Salvage therapy of aggressive lymphomas[a]

Patients	Patients who achieved complete response or >90% partial response			
	ProMACE	Etoposide+ cisplatin	6-MP	Overall
Relapsed ($n = 26$)	11/26 (42%)	4/13 (31%)	1/6 (17%)	16/26 (62%)
Induction failures ($n = 16$)	—	3/16 (19%)	0/4 (0%)	3/16 (19%)

Total No. of patients who received transplants	19 (45% of aggressive treated) (37% of potentially eligible)
No. of relapses after transplant	7 (37%) all within 6 mo
Fraction of potentially eligible who may benefit from aggressive therapy	12/51 (24%)

[a] Of 51 potential candidates, 9 (18%) were not treated aggressively (4 because of medical contraindications and 5 because of refusal); 42 (82%) were treated aggressively.

to respond sufficiently to cisplatin plus etoposide were offered treatment with high-dose 6-mercaptopurine. Overall, 62% (16 of 26) of the patients who had relapsed obtained a sufficient response to take to transplantation, and 19% (3 of 16) of the induction failures received transplants. Of the 19 patients undergoing high-dose cyclophosphamide followed by total-body irradiation, 7 patients relapsed, all within the first 6 months. Thus the fraction of potentially eligible patients (i.e., all who relapsed and induction failures) who benefited from the aggressive treatment was 12 of 51, or 24%. This is a rather promising development for a group of patients once thought to have a uniformly fatal outcome. From the durability of the responses after bone marrow transplantation (one-half or more of the patients stay in remission), there is reason for us to believe that the addition of high-dose therapy to the conventional-dose salvage programs, such as DHAP, MIME, ProMACE, and others, will improve the survival.

The results with bone marrow transplantation have been so encouraging that some investigators, notably Gulati et al. at Memorial Sloan-Kettering Cancer Center (*63*), have used high-dose therapy with bone marrow support as the primary treatment of patients with poor prognostic factors. Gulati and co-workers have obtained long-term survival in about 70% of the patients with large mediastinal or abdominal masses or high lactate dehydrogenase levels by using high-dose cyclophosphamide and total-body irradiation as primary treatment. This group of patients had a long-term survival of 20% in their previous experience. Clearly, this represents an extension of the principle of optimizing the dose intensity of treatment. The use of bone marrow or peripheral stem cell (*64*) transplantation or colony-stimulating factors in support of high-dose chemotherapy programs represents the next phase of development of primary treatment programs for selected subsets of patients who have a lower probability of cure with the maximum tolerated doses of existing combinations. It is our hope that the development of new information on the biology and gene regulation of lymphoma cells may reveal completely new targets for therapeutic intervention in the near future.

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Salvage Therapy for Patients With Non-Hodgkin's Lymphoma

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ABSTRACT—The non-Hodgkin's lymphomas represent a diverse group of lymphoproliferative disorders for which treatment must be specified according to the patient's status as well as the disease status. Although many advances have been made in the front-line treatment of non-Hodgkin's lymphomas, more than 50% of the patients will not be cured with their initial therapy. Because of these treatment failures with front-line therapy, many different salvage therapies have been tried in this patient population. In this article, we describe several conventional-dose, salvage chemotherapies and strategies for dose escalation and hematopoietic stem cell transplantation in an attempt to overcome chemotherapy resistance.—J Natl Cancer Inst Monogr 10:39–43, 1990.

Non-Hodgkin's lymphomas include a number of specific malignant neoplasms that can be cured with chemotherapy. Burkitt's lymphoma (i.e., small noncleaved cell lymphoma) was among the first tumors to be shown to be curable with cytotoxic chemotherapy (*1*). The most common aggressive non-Hodgkin's lymphomas, usually called diffuse large cell lymphomas, have been known to be curable since the reports from Yale in 1972 (*2*) and the National Cancer Institute in 1973 (*3*). Since those initial reports, the addition of new agents and the development of new combinations of drugs have resulted in a wide variety of regimens with curative potential in the aggressive non-Hodgkin's lymphomas. Unfortunately, all patients are not cured. At least 50% of the patients with diffuse large cell lymphoma will not survive their illness with the presently available treatment approaches, and higher proportions of patients with other subtypes of non-Hodgkin's lymphoma are not cured. At the present time, the issue of therapy after relapse (i.e., salvage therapy) is a major consideration for oncologists who manage patients with non-Hodgkin's lymphoma. There is no one correct approach to salvage therapy in patients with relapsed non-Hodgkin's lymphoma. The wide variety of issues that the clinician must consider in planning salvage therapy for any particular patient is listed in table 1.

It is quite clear that very young patients are more likely to be managed aggressively than are the elderly patients. For example, bone marrow transplantation would not be an option for most patients over 60 years of age. The patient's general health will limit the use of certain salvage therapies. For example, even a young patient with a low performance status or serious heart, renal, or pulmonary disease might not be a candidate for the most intensive salvage therapies. The histologic subtype of lymphoma is also an important consideration. Most of the discussion in this report will be regarding the salvage therapy for patients with aggressive non-Hodgkin's lymphoma. However, most patients with the indolent type will relapse and be candidates for salvage therapy. Also, the long natural history of this illness leads most oncologists to adopt a less aggressive approach to the subsequent therapy they prescribe. When the patient is a candidate for bone marrow transplantation, the presence or absence of bone marrow involvement might dictate the therapeutic options. For example, a patient with significant bone marrow involvement is not likely to be a candidate for autologous bone marrow transplantation. Patients with disease that remains chemotherapy sensitive after relapse have a much better outlook with bone marrow transplantation than do those who have become absolutely chemotherapy resistant (*4*). The types of therapy the patient has previously undergone may dictate choices for salvage therapy. For example, a patient who has had extensive thoracic radiation will not be a candidate for total-body radiotherapy. Finally, the most important consideration is often the reported chances for success of the available salvage treatments.

SALVAGE THERAPY AFTER INITIAL TREATMENT FOR LOCALIZED DISEASE

Patients with non-Hodgkin's lymphoma are rarely treated with only surgery. However, in those unusual circumstances when the patient is operated on for localized extranodal disease and subsequently relapses, there is no evidence that the patient who receives aggressive chemotherapy regimens has less chance for success than does one who initially presents with disseminated disease. Patients who present with localized non-Hodgkin's lymphoma are sometimes treated only with radiotherapy. When these patients relapse and are then treated with chemotherapy, the results may not be as good as those seen in Hodgkin's disease in the same clinical situation. Patients with Hodgkin's disease who are treated initially with radiotherapy and then relapse and receive chemotherapy seem to have an especially good outlook (*5*). In one series in which 15 patients with diffuse, aggressive non-Hodgkin's lymphoma received cyclophosphamide–doxorubicin–vincristine–prednisone (CHOP) after relapse following radiation therapy for localized disease, the complete remission rate was only 33%, and only 20% of the patients were long-term survivors (*6*). When these patients were compared with 62 patients treated at the same institution with the above regimen for de novo diffuse non-Hodgkin's lymphoma, the results were significantly better (i.e., a complete remission rate of 66% and a long-term survival rate of 45%) than were those of patients who were treated after they failed radiotherapy. In light of the excellent results reported with combined modality therapy or aggressive chemotherapy in patients with localized non-Hodgkin's lymphoma, it does not seem appropriate for these patients to be treated only with a local treatment modality, such as surgery or radiation therapy, except in unusual circumstances (*7*).

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Table 1.—Factors for consideration in planning salvage therapy for patients with non-Hodgkin's lymphoma

Patient age
General health (performance status)
Other significant illnesses (i.e., major organ dysfunction)
Histopathology
Sites of disease
Marrow status
Response to previous therapy
Types of previous therapy
Success rates of available salvage therapies

Table 3.—Response to cisplatin-based salvage chemotherapy regimens in 58 patients[a]

Previous chemotherapy response	No. of patients	Complete responses	
		No.	Percent
Complete response ≥ 12 mo	13	3	23
Complete response < 12 mo	21	3	14
No complete response	24	1	4

[a]Data are from the Nebraska Lymphoma Study Group.

RESULTS AFTER FAILURE OF PRIMARY CHEMOTHERAPY REGIMENS

Few data are available regarding the results of aggressive cytotoxic chemotherapy regimens in patients who fail what would now be considered nonaggressive chemotherapy such as cyclophosphamide–vincristine–prednisone (CVP). The scarce data reported in the literature (*8*) suggest that exposure to any chemotherapy markedly decreases the results with subsequent aggressive regimens. Thirty-three patients treated initially with the three-drug combination above and who then received doxorubicin-based regimens had a complete remission rate of only 15%.

A wide variety of salvage chemotherapy regimens has been reported in patients with relapsed or refractory non-Hodgkin's lymphoma. Table 2 lists several series in which at least 20 patients were reported. As can be seen, a high rate of complete responses is not shown for most regimens, and most of those that are observed are not durable. The largest experience has been by investigators at the M.D. Anderson Cancer Center (Houston, Tex.) using the mitoguazone–ifosfamide–methotrexate–etoposide (MIME) regimen. Although the complete response rate was approximately 30%, only 20% of the complete responders were long-term, disease-free survivors. Thus the potential cure rate was less than 10%. More recently, the dexamethasone–high-dose cytarabine–cisplatin (DHAP) regimen has been widely used for patients with non-Hodgkin's lymphoma who relapsed. Although the complete response rate has been as high as 30%, the proportion of long-term disease-free survivors has not been described. In Hodgkin's disease, the chances for a patient to achieve a complete and durable remission after relapse seem to be related to the duration of the initial response to chemotherapy. Patients with a brief initial remission do less well than do patients who have very long remissions (*17*). These same rules seem to apply for the use of salvage chemotherapy in non-Hodgkin's lymphoma. Table 3 presents the results with cisplatin-based salvage chemotherapy regimens in 58 patients treated by oncologists from the Nebraska Lymphoma Study Group. Patients who were treated with salvage chemotherapy after initial remission lasting at least 12 months had a 23% complete remission rate, in contrast to a 14% complete remission rate in patients who relapsed after a shorter complete remission. Patients who had never been in complete remission had only a 4% complete response rate to salvage chemotherapy.

The studies summarized above demonstrating the unsatisfactory results with salvage therapy for non-Hodgkin's lymphoma with chemotherapeutic agents administered at conventional doses are particularly disappointing, in light of the success with front-line therapy. In general, complete responses with salvage

Table 2.—Results with salvage chemotherapy in non-Hodgkin's lymphoma

Regimen	No. of patients evaluated	Complete responses	No. alive in complete remission	Reference
Mitoguazone + ifosfamide + methotrexate + etoposide	123	32	Twenty percent of complete responders long-term, disease-free survivors with diffuse large cell lymphoma	(*9*)
Ifosfamide + methotrexate + etoposide	41	5	4	(*10*)
Ifosfamide + methotrexate + etoposide	38	15	Median relapse-free survival for complete responders of 12 mo	(*11*)
Cytarabine + cisplatin + etoposide	27	2	1	(*12*)
Mitoguazone + etoposide + gallium nitrate	27	5	2	(*13*)
Etoposide + mitoguazone	24	1	0	(*14*)
Teniposide + cytarabine + cisplatin	24	2	1	(*15*)
Cytarabine + cisplatin + dexamethasone	83	28	8 (with 11-mo median follow-up)	(*16*)

Table 4.—Bone marrow transplantation in non-Hodgkin's lymphoma

Type of transplant	No. of patients	Alive in complete remission		Treatment-related deaths	
		No.	Percent	No.	Percent
Allogeneic	89	44	49	27	30
Syngeneic	9	4	44	0	0
Autologous	535	193	36	74	14

therapy have been unusual, and long-term, disease-free survival was reported only in a small proportion of patients. The addition of new agents may alter these results. However, at the present time, the most hopeful information has come from the use of dose escalation to overcome chemotherapy resistance in these patients with lymphomas who have relapsed.

BONE MARROW TRANSPLANTATION

Bone marrow transplantation offers oncologists the possibility to circumvent treatment resistance by increasing the dose of available cytotoxic agents and radiotherapy while ameliorating myelotoxicity by infuson of hematopoietic stem cells. One would expect this treatment approach to be successful in some patients if a dose response to therapy exists. Appelbaum et al. (*18*) first reported long-term disease-free survival with high-dose chemotherapy and autologous bone marrow transplantation in children with relapsed Burkitt's lymphoma. Since that time, various studies have been published on variable high-dose regimens followed by infusion of hematopoietic stem cells. The hematopoietic stem cells have most commonly been obtained from the patient being treated (i.e., autologous bone marrow transplantation), but series in which investigators used HLA-matched sibling donors (i.e., allogeneic bone marrow transplantation) or hematopoietic stem cells from identical twins (i.e., syngeneic transplantation) have also been reported. Table 4 lists the summarized results of these three sources of hematopoietic stem cells. As can be seen, allogeneic transplantation seems to be associated with a higher treatment-related death rate. However, the net result of therapy does not seem to vary widely regardless of the source of hematopoietic stem cells. This is supported by a report from investigators in Seattle (*19*), who did not find a significant difference among patients receiving transplants for lymphoma regardless of the source of hematopoietic stem cells. Because the results are much more extensively reported for the use of autologous bone marrow transplantation, most of our remaining comments about transplantation refer to those series in which autologous hematopoietic stem cells were used. The frequency of autologous bone marrow transplantation has markedly increased in the last few years (*20*). The disease most frequently treated has been non-Hodgkin's lymphoma.

The most common disease reported to be treated with autologous bone marrow transplantation has been diffuse large cell lymphoma. Philip et al. (*4*) demonstrated that it is possible to predict outcome for high-dose therapy and autologous bone marrow transplantation in patients with diffuse large cell lymphoma based on the responsiveness of the tumor to salvage chemotherapy administered at traditional doses. One hundred patients treated with high-dose therapy and autologous bone marrow transplantation from centers in Europe and America were analyzed. Patients who had never been in complete remission had particularly poor prognoses. Only 9 of 34 patients (26%) achieved a complete remission, and none of these patients were long-term, disease-free survivors. Ten of 22 (45%) patients who had been in complete remission but whose tumors had become resistant to chemotherapy achieved complete remission, but only 14% of the patients achieved long-term, disease-free survival. Forty of 44 patients (91%) who had relapsed from a complete remission and still had chemotherapy-sensitive disease (i.e., they achieved a complete or partial response with salvage chemotherapy at regular doses) had complete responses. More importantly, 36% of these patients were long-term, disease-free survivors.

The response to previous chemotherapy was highly significant in predicting outcome to the autologous bone marrow transplant and, in a multivariate analysis, was the only significant variable. These results have recently been confirmed in a prospective study (*21*). Fifty patients relapsing from complete remission were treated with the dexamethasone–high-dose cytarabine–cisplatin regimen; of these patients, 58% had a complete or partial response. Twenty of the patients then underwent autologous bone marrow transplantation. Following the transplant, the complete response rate was 85%, and 50% of all patients were alive in complete remission at the time of the report. The early death rate related to the bone marrow transplant in this series was 10%. In the other 9 patients, the transplant could not be performed or the patients refused. This provides some insight into the actual ability of physicians to apply autologous bone marrow transplantation to patients with relapsed lymphoma. The selection factors involved in the decision as to which patients will receive transplants (the transplant itself can be an obstacle) results in some patients failing to undergo the treatment despite the *intention* to do so and makes published percentages of "success" difficult to interpret.

No one has completed a randomized trial comparing salvage chemotherapy at regular doses and autologous bone marrow transplantation in the treatment of patients with relapsed non-Hodgkin's lymphoma. However, such a study is currently under way (*21*). An indirect answer to this question is available from the results of a series of patients with relapsed lymphomas (treated by physicians of the Nebraska Lymphoma Study Group) who underwent autologous bone marrow transplantation or cisplatin-based salvage chemotherapy (*22*). Between 1983 and 1987, 17 patients were referred for autologous bone marrow transplantation; they were 60 years of age or less and in first relapse following a chemotherapy regimen that included doxorubicin. By chance, there were also 17 patients with the same characteristics who were treated with a cisplatin-based salvage chemotherapy regimen. These findings were not the result of a randomized prospective trial, but the patients received the treatment they or their personal oncologist favored. However, no patients who were considered for autologous bone marrow transplantation and rejected were then counted in the chemotherapy group. The results of these patients are presented in table 5. The patients undergoing autologous bone marrow transplantation had far more favorable results. In fact, since the onset of the Nebraska Lymphoma Study Group, we have

Table 5.—Autologous bone marrow transplant vs. cisplatin-based chemotherapy in patients ≤ 60 yr old in first relapse[a]

Characteristic	Bone marrow transplant	Cisplatin
Age, yr	50[b]	56[c]
Male, %	71	59
Diffuse large cell lymphoma, %	71	53
Complete remission, %	65	18
Three-year disease-free survival, %	40	0

[a]Seventeen patients received the autologous bone marrow transplant and 17 received cisplatin.
[b]Ages ranged from 15 to 59 yr.
[c]Ages ranged from 15 to 60 yr.

observed no long-term, disease-free survivors in patients treated only with salvage chemotherapy at traditional doses.

The most hopeful approach for improving results in autologous bone marrow transplantation for patients with diffuse large cell lymphoma lies in oncologists treating patients at the optimal time in the course of their illness. When it has been successfully applied, the experience with autologous bone marrow transplantation in all diseases has been that patients treated early in the course of their illness, i.e., at a time of minimal, chemotherapy-sensitive disease, have far superior results. Gulati et al. (*23*) reported the results of autologous bone marrow transplantation in 31 patients with diffuse large cell lymphoma, 14 of whom received the transplant as part of their initial therapy for poor-prognosis, B-cell, diffuse large cell lymphoma with a large mediastinal mass. Seventeen similar patients received a transplant only after failing a front-line chemotherapy regimen. Eleven (79%) of the patients treated as part of their primary therapy were alive and well at the time of the report in contrast to only 4 (24%) of the patients treated later in the course of their disease. Philip et al. (*24*) reported that patients treated in partial remission to their initial chemotherapy regimen before progressing have a result similar to that reported by Gulati. Although patients who have failed to achieve initial complete remission promptly might well be candidates for early transplantation, those who achieve a complete remission who would be good candidates for bone marrow transplantation are difficult to identify. A significant proportion of all groups of complete responders will be cured without subsequent therapy. Until better ways are available to identify patients with a particularly poor outlook, bone marrow transplantation in first complete remission is probably not an appropriate therapeutic choice for patients with diffuse large cell lymphoma.

Bone marrow transplantation before relapse might be appropriate in certain subgroups of patients with high-grade non-Hodgkin's lymphoma. Patients with Burkitt's lymphoma (*25*) and lymphoblastic lymphoma (*26*) who present with bulky disease and high lactate dehydrogenase levels have a very poor outlook with traditional chemotherapy. However, these patients usually achieve an initial complete response. These subgroups of patients might well be treated with high-dose therapy and autologous bone marrow transplantation at best initial response. Data from the European Bone Marrow Transplant Group suggest that such patients have an excellent outlook when they receive transplants in their first complete remissions (*27*).

Patients with follicular non-Hodgkin's lymphoma generally have a long survival despite frequent relapses. These patients regularly respond to salvage chemotherapy at traditional doses, but cures are extraordinarily unusual. Recently, autologous bone marrow transplantation has been studied in these patients. We have treated 21 such patients with relapsed follicular lymphoma at the University of Nebraska Medical Center. These patients had a median age of 40 years and had received a median of two prior chemotherapy regimens. The disease of 9 patients was called follicular, small cleaved cell non-Hodgkin's lymphoma, and 12 had follicular mixed cell lymphoma. Fifteen of 20 patients (75%) who could be evaluated were in complete remission after they had undergone the transplant procedure; 13 (65%) have remained in complete remission for 3–24 months (September 1989). The follow-up is obviously much too short for one to make a determination about whether this treatment approach will result in long-term, disease-free survival for a proportion of patients with relapsed follicular lymphoma.

CONCLUSIONS

Although a subgroup of patients with non-Hodgkin's lymphoma can be cured with primary chemotherapy regimens, most will eventually be candidates for some form of salvage therapy. We have illustrated the poor results currently achieved with such treatments. At the present time, the best reported results have been with dose escalation requiring autologous bone marrow transplantation to alleviate the associated myelosuppression. However, this treatment approach is associated with a high treatment-related morbidity and mortality and is very expensive. Even with improvements in our ability to perform bone marrow transplantation or in the appearance of new chemotherapeutic agents to allow more effective salvage therapy using regimens at traditional doses, approaches to salvage treatment are likely to remain unsatisfactory. This illustrates clearly the importance of optimal staging and meticulous primary therapy for patients with non-Hodgkin's lymphoma. It is far preferable for patients to be cured with their primary therapy rather than have to deal with the difficult issues associated with salvage treatments.

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Acquired Immunodeficiency Syndrome-Associated Lymphomas

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ABSTRACT—Congenital and acquired states of immunodeficiency have long been associated with an increased incidence of malignant lymphoma. An increased incidence of non-Hodgkin's lymphomas was recognized early in the epidemic immunodeficiency state associated with the human immunodeficiency virus (HIV) infection AIDS. Although the precise etiologic mechanism of these lymphomas remains speculative, the presence of Epstein-Barr viral proteins or sequences and characteristic chromosomal translocations giving rise to altered expression of the c-myc oncogene have frequently been observed. It has been suggested that HIV infection leading to disordered T-lymphocyte function (possibly in conjunction with Epstein-Barr infection) leads to the emergence of polyclonal populations of stimulated B lymphocytes. These cells, which undergo physiologic immunoglobulin gene rearrangement, may provide the background for the occurrence of characteristic chromosomal translocations that lead to the emergence of malignant lymphomas. These lymphomas tend to present clinically with high-grade histopathologic subtype, advanced stage, and a propensity for the involvement of otherwise unusual extranodal sites, including the central nervous system. The experience with therapy for HIV-associated lymphomas has indicated that highly aggressive, dose-intensive chemotherapy regimens may be associated with inferior results. More recent regimens have stressed less myelosuppressive therapy combined with prophylaxis for central nervous system disease and pneumocystis infection. The dominant prognostic factors in the HIV-associated lymphomas appear to be primarily related to the underlying HIV infection and include total CD4 lymphocyte count, performance status, and prior AIDS diagnosis.—J Natl Cancer Inst Monogr 10:45–54, 1990.

BACKGROUND

It has long been recognized that congenital and acquired states of immunodeficiency are associated with an increased incidence of malignant lymphoma. Recipients of organ allotransplants on immunosuppressive therapy have a greater than 100-fold increased risk of developing a de novo cancer (*1, 2*), and most of these malignant neoplasms will be of lymphoreticular origin. Indeed, when the incidence of lymphomas alone is examined in kidney transplant recipients, there is a 350- to 700-fold increased incidence compared with normal age-matched individuals (*3*). Patients undergoing renal, cardiac, or bone marrow allotransplantation are all clearly at increased risk for developing lymphoma (*3–6*). Transplant-related lymphomas may present in a wide variety of anatomic sites, and isolated CNS presentations are common (*7*).

Acquired autoimmune disorders represent another setting in which there is a documented increase in lymphoproliferative cancers. Collagen vascular diseases (*8*), Sjögren's or sicca syndrome (*9–11*), and immunoblastic lymphadenopathy with dysproteinemia (*12*) represent examples of such conditions associated with an increased incidence of lymphoma.

Congenital immunodeficiency states are also strongly associated with excess incidence of malignant lymphomas and include Wiscott-Aldrich syndrome (*13*), X-linked proliferative disorder in association with EBV (*14*), severe combined immunodeficiency (*15*), ataxia-telangiectasia (*15, 16*), Bruton's hypogammaglobulinemia (*17*), common variable immunodeficiency (*18*), and Chédiak-Higashi syndrome (*19*).

Recurrent themes in the presentation and clinical behavior of these malignant lymphomas occurring in immunodeficiency are B-cell phenotype, high-grade histologic subtype, rapid clinical progression, involvement of extranodal and unusual anatomic sites, and prominent CNS and bone marrow involvement. With this background, it is therefore not surprising that the lymphomas occurring in the epidemic immunodeficiency state AIDS recapitulate many of these same clinical characteristics.

EPIDEMIOLOGY

In 1981–1982, the first cases of AIDS were reported by the Centers for Disease Control (*20*). In 1982, the Centers reported the first four cases of NHL in homosexual males (*21*). In 1985, the appearance of high-grade B-cell lymphomas was included in the Centers' diagnostic criteria for AIDS (*22*). The first large multicenter study of NHL in 90 homosexual men was published by Ziegler et al. in 1984 (*23*), and since that time numerous studies on AIDS-associated NHL have been published (*24–32*).

Estimates of the incidence of Hodgkin's disease and NHL among HIV-infected persons are plagued with problems of selected populations, retrospective analysis, and short follow-up. Nevertheless, most investigators have observed a 5%–10% incidence of NHL among persons infected with HIV (*33, 34*). Biggar and co-workers (*35*) used the population-based registry of the Surveillance, Epidemiology, and End Results Program to calculate a significant increase in the morbidity odds ratio for high-grade lymphomas in the San Francisco metropolitan area

ABBREVIATIONS: EBV = Epstein-Barr virus; AIDS = acquired immunodeficiency syndrome; NHL = non-Hodgkin's lymphoma; HIV = human immunodeficiency virus; CNS = central nervous system; NCI = National Cancer Institute; MOPP = mechlorethamine–vincristine–procarbazine–prednisone; ABVD = doxorubicin–bleomycin–vincristine–dacarbazine; CVP = cyclophosphamide–vincristine–prednisone; CHOP = cyclophosphamide–doxorubicin–vincristine–prednisone; COMP = cyclophosphamide–vincristine–methotrexate–procarbazine or prednisone; BACOP = bleomycin–doxorubicin–cyclophosphamide–vincristine–prednisone; M-BACOD = high-dose methotrexate–bleomycin–doxorubicin–cyclophosphamide–vincristine–dexamethasone; COMLA = cyclophosphamide–vincristine–methotrexate–leucovorin–cytarabine; ProMACE/MOPP = prednisone–methotrexate–doxorubicin–cyclophosphamide–etoposide + MOPP; AZT = zidovudine; CSF = colony-stimulating factor; GM-CSF = granulocyte/macrophage-CSF.

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to compare the preepidemic period 1973–1980 with 1981–1982 (odds ratio, 9.1; 95% confidence interval, 1.8–45.6). In a separate study conducted in the San Francisco Bay Area, Harnly et al. (*36*) reported that the pattern of increased incidence of NHL was similar to that of Kaposi's sarcoma in an index population-at-risk for AIDS. These investigators also noted a fivefold increase in the incidence of NHL in this population compared with pre-AIDS epidemic rates. Notably, no other tumors possibly related to AIDS, including Hodgkin's disease, occurred with increased incidence rates. In a study of prisoners and other iv drug abusers, Ahmed and associates (*37*) reported a 6- to 18-fold higher incidence of NHL among all New York State prisoners aged 20–49 years, with a 40-fold increased incidence rate among those who were iv drug abusers. With a national incidence rate of NHL of 30,000/year in persons not at risk for HIV infection, and a conservative estimate of 1–2 million HIV-infected persons in the United States with a lifetime risk of 5%–10% of developing NHL, it is clear that AIDS-associated lymphomas will constitute an increasingly large proportion of all newly diagnosed NHL.

Hodgkin's disease occurring in the setting of AIDS has been described in a number of series and case reports (*26, 27, 30, 37–46*), but convincing results of epidemiologic studies demonstrating an increased incidence of Hodgkin's disease in persons-at-risk for AIDS are lacking. An increased incidence of Hodgkin's disease has been suggested among iv drug abusers in the New York prison study of Ahmed et al., but this is based on 4 cases (*47*). Nonetheless, the clinical characteristics and prognosis for Hodgkin's disease associated with AIDS are markedly different from those for Hodgkin's disease in the non-AIDS population. Further epidemiologic studies are needed to clarify the relationship between AIDS and Hodgkin's disease. Several other lymphoproliferative disorders have been described that may be related to AIDS, but causal relationships remain conjectural at present.

PATHOLOGY

A striking feature of the AIDS-associated NHL is a unique distribution of histologic subtypes. Table 1 is a compilation of histologic subtypes for the AIDS-associated NHL accrued in four large reported series and includes only patients whose tumors could be assigned a "Working Formulation" classification (*48*). Remarkably, more than one-half to three-fourths of these patients presented with lymphomas of high-grade histology, evenly distributed between small noncleaved (diffuse undifferentiated Burkitt's and non-Burkitt's), and large cell, immunoblastic histologies. Intermediate-grade histologies (predominantly diffuse large cell) form the bulk of the remaining AIDS-associated NHL, with low-grade histologic subtypes being rare. The predominance of the small noncleaved subtype in most of these series is particularly noteworthy, because this diagnosis ordinarily accounts for, at most, 10% of the NHL in the United States (*21*).

Table 1.—Working Formulation histologic subtypes of AIDS-associated NHL in four reported series

Classification	No. of patients with tumors[a]			
	Series in reference:			
	(*23*)	(*30*)	(*31*)	(*32*)
High grade				
Small noncleaved	32	16	36	29
Large cell, immunoblastic	22	8	25	36
Lymphoblastic	2			
Total, %	63	56	69	78
Intermediate grade				
All histologies	26	16	28	18
Total, %	30	37	31	22
Low grade				
All histologies	6	3	0	0
Total, %	7	7	0	0

[a] Only those patients whose tumors could be assigned a Working Formulation classification were included.

The majority of AIDS-associated NHL are of B-cell origin, by virtue of expression of the B-cell-restricted antigen B1, expression of surface immunoglobulin, and lack of expression of T-cell-associated antigens (*31*). The small noncleaved lymphomas tend to be surface immunoglobulin positive, C3d receptor negative, and common acute lymphocytic leukemia antigen positive. The large noncleaved cell subtype heterogeneously expresses surface immunoglobulin, C3d receptor, and the acute lymphocytic leukemia antigen positive (*31*). Knowles et al. (*31*) have demonstrated that the AIDS-associated NHL examined in their series have immunoglobulin heavy-chain rearrangements, frequently with multiple rearrangements in the same tumor, but with no T-cell receptor (T_β) rearrangements. As suggested by these investigators, such data indicate that the AIDS-associated B-cell NHL are immunophenotypically indistinguishable from the B-cell NHL not associated with AIDS (*31, 49, 50*), but they may share the unique feature of polyclonal B-cell expansion with other lymphomas arising in immunodeficiency states (*50–52*).

Hodgkin's disease occurring in AIDS patients has an increased incidence of mixed cellularity and lymphocyte-depleted histologies in some series (*46*). Because most of the lymphocytic infiltrate in Hodgkin's disease is probably composed of normal reactive lymphocytes, it is unclear whether a possible increase in the lymphocyte-depleted histologic subtype is related to the lymphopenia induced by cytopathic HIV, or whether some more complex mechanism is involved. Some investigators (*31, 53*) have reported decreased total numbers of T cells and an inverted T4:T8 ratio in nodal tissue of some patients with AIDS-associated Hodgkin's disease. Immunophenotypic and molecular genetic analysis has otherwise not revealed significant differences between the AIDS-related and -nonrelated Hodgkin's disease.

Other lymphomas have been described in AIDS patients or others at risk for AIDS. Knowles et al. (*31*) described 3 patients with chronic lymphocytic leukemia and a peripheral cytotoxic T-cell phenotype. Antigen-receptor gene rearrangement analysis in 2 of these patients indicated that their leukemias arose from a clonal expansion of a mature T3+, T4−, T8+ suppressor cytotoxic T-cell subset. These investigators (*50*) have presented data that suggest the clonal integration of human T-cell leukemia virus-1 genomic sequences into the tumor cells of 1 of these patients. Gallo et al. (*54*) had previously reported isolation of this same virus in an AIDS patient with a cerebral lymphoma. A patient with cutaneous T-cell lymphoma (mycosis fungoides) with Sézary syndrome, and 4 patients with intermediate-grade large cell lymphomas, some of which had

immunophenotypic features suggestive of peripheral T-cell lymphoma, were described by Kaplan and his associates (*32*). Plasmacytoma progressing to multiple myeloma (*30*), frank multiple myeloma (*29*), B-cell acute lymphoblastic leukemia (*55*), and T-cell lymphoblastic lymphoma (*56*) have also been described in patients with AIDS.

ETIOLOGY

The HIV does not appear to have a direct etiologic role in AIDS–NHL, and the lack of integration of HIV genomic sequences into the hyperplastic lymphoid tissue of patients with AIDS-related complex or the tumor cells of those with AIDS–NHL supports this view (*50*). However, it is clear that HIV infection results in major alterations in B-cell function characterized by polyclonal B-cell activation that leads to spontaneous immunoglobulin secretion, hypergammaglobulinemia, and lymphoid hyperplasia (*57*). In this altered environment of polyclonal B-cell activation, other processes, including EBV infection and certain oncogenetic alterations closely related to the normal physiologic process of B-cell immunoglobulin gene rearrangements, are believed to be related to the etiology of the AIDS–NHL.

Many investigators have speculated that the EBV is at least a cofactor in the oncogenesis of AIDS-related NHL. Several (*58–60*) have strongly implicated a role for EBV in the genesis of transplant-related NHL. Active EBV infection is common in male homosexuals and iv drug abusers who are subsequently diagnosed as having AIDS (*61*). Tumor specimens from endemic African Burkitt's lymphoma uniformly contain the EBV genome and viral proteins, particularly the EBV-induced nuclear antigen (*62*). These Burkitt's lymphomas exhibit reciprocal chromosomal translocations involving portions of chromosomes containing immunoglobulin genes (chromosome 2, kappa light chain; chromosome 14, immunoglobulin heavy chain; chromosome 22, lambda light chain) and chromosome 8, which contains the c-myc oncogene (*63*). Such translocations, of which the t(8;14) is the most common, result in placement of the c-myc oncogene in proximity to the transcriptional regulatory control elements of immunoglobulin genes giving rise to altered c-myc expression (*63, 64*). This abnormal c-myc expression is believed to be related to malignant transformation resulting in a clonal population of tumor cells by mechanisms that are not completely understood. Indeed, Lombardi et al. (*65*) have demonstrated tumorigenic conversion of EBV-infected human B lymphocytes with the introduction of activated c-myc oncogene. Characteristic c-myc gene rearrangements and translocations have been observed frequently in AIDS-associated B-cell NHL (*66–68*). An AIDS-associated plasma cell cancer containing EBV genomic material has also been described (*69*), and 7 of 9 cases of oral AIDS-associated NHL have been reported to contain EBV sequences by in situ hybridization (*70*).

The presence of 30–100 copies of the EBV genome per cell was determined in a primary AIDS-associated NHL of the CNS (*71*). In addition, Baumgartner and co-workers (*72*) found that 6 of 6 tumor samples from primary AIDS-related lymphomas of the CNS did not contain c-myc rearrangements, but 5 of 5 tested tumors had EBV sequences detected. This latter finding suggests that EBV has an effect in AIDS-associated neoplasia. In 3 cases of unclassifiable, pleomorphic AIDS-associated neoplasms, B-cell lineage was demonstrated by clonal immunoglobulin heavy- and light-chain rearrangements, but none had c-myc rearrangements, and all contained EBV proteins or sequences, or both (*73*).

However, a direct etiologic role for EBV in the AIDS-associated NHL has been questioned by Kaplan et al. (*32*) who were unable to demonstrate evidence of EBV genomic sequences in 10 of 15 evaluated lymphomas. Similarly, in 16 cases of AIDS-associated NHL, Subar and co-workers (*74*) found 12 with c-myc gene rearrangements and only 6 with detectable EBV sequences or proteins. Feigal and associates (*75*) reported that one NHL tumor focus obtained at autopsy in a patient who had AIDS contained a single-sized EBV terminus, which indicated that this tumor represented a clonal expansion of an EBV-infected progenitor, whereas a separate tumor focus contained no detectable EBV sequences. These observations would suggest that either EBV infection or altered c-myc expression is associated with at least some forms of AIDS-associated lymphoid neoplasia. It would also appear that c-myc translocation and EBV infection are not always linked in an obligate fashion in the process of AIDS-associated lymphomagenesis, although they may have a combined role in at least a subset of AIDS–NHL.

The etiologic mechanism for the AIDS–NHL, if there is a dominant one, remains speculative. It is clear that the HIV-infected substratum for lymphoid neoplasia includes chronic B-cell stimulation with lymphoid hyperplasia, disordered regulatory T-cell function, and the emergence of polyclonal populations of B-cell clones, as Knowles et al. (*50*) have suggested. Chronic EBV infection may also contribute to this polyclonal stimulation. Several investigators have proposed that, in this environment, B cells may undergo neoplastic transformation with c-myc translocations resulting in deregulated c-myc expression. Such altered c-myc expression results from genetic errors, whose probability is increased in a hyperstimulated population of cells undergoing physiologic immunoglobulin gene rearrangements, and may ultimately result in the emergence of a clonal population of malignant lymphocytes.

CLINICAL CHARACTERISTICS

The population in which the diagnosis of AIDS-associated NHL is made in the United States has been largely homosexual males with smaller numbers of iv drug users, transfusion-related cases, and female sexual partners of male HIV-infected individuals (*23, 25–32*). Other populations, as described in a recent Italian series, in which the majority of patients had a history of iv drug use as the primary AIDS risk factor, have also been reported (*43*).

The AIDS diagnostic status at the time the diagnosis of NHL is made may be changing over time and is a significant prognostic factor. In Ziegler's original series, 47% of the patients with AIDS–NHL had a previous diagnosis of AIDS with Kaposi's sarcoma, an opportunistic infection, or both (table 2). Of the patients with AIDS–NHL, 37% had AIDS-related complex, and only 15% of the patients had no previous AIDS-related diagnosis. In the more recently reported San Francisco series, the proportion of patients with no preceding AIDS-related diagnosis was 31%, with the proportions of patients with frank AIDS or the related complex being 27% and 25%, respectively. Indeed, among the 12 AIDS–NHL patients recently treated over the past 2 years at the NCI and Lombardi

Table 2.—AIDS diagnostic status at time of diagnosis of AIDS-NHL

Previous AIDS diagnosis, %			
AIDS	AIDS-related complex	None	Reference
47	37	15	(*23*)
27	25	31	(*32*)
25	16	58	[a]

[a] Values are from my unpublished observations of patients treated at the NCI and Lombardi Cancer Center.

Table 3.—Stage and extranodal involvement at presentation of AIDS-NHL patients

Stage, %			
I, II	III, IV	Extranodal sites, %	Reference
42	58	98	(*23*)
36	64	87	(*31*)
26	74	65	(*30*)
16	82	74	(*32*)
17	83	91	[a]

[a] *See* footnote, table 2.

Cancer Center, 58% had no antecedent AIDS-related diagnosis, and 25% and 16% were in the AIDS and related complex categories, respectively (unpublished observations). The reasons for these differences are not entirely clear, but it must be recognized that changes in the AIDS population with respect to risk factors, possible earlier recognition of HIV infection status, and impact of antiretroviral therapy on the natural history of the disease may all interact to alter the underlying clinical setting in which the AIDS-associated NHL will be diagnosed in the future.

The most striking clinical characteristics of the AIDS-associated NHL include presentation with advanced-stage disease frequently involving extranodal anatomic sites. The Ann Arbor stage distribution for several large reported series and the author's series of 12 patients is shown in table 3. Between 64% and 83% of the patients present with stage III or IV disease, with between 65% and 91% having extranodal disease at the time of presentation. The four most common extranodal sites in all reported series are bone marrow, CNS (parenchymal or meningeal), liver, and gastrointestinal tract (*23, 25, 27, 28, 30–32*). The AIDS-associated NHL are also remarkable for extranodal involvement of other unusual anatomic locations; table 4 contains a listing of such sites reported in several large series. In this group, pulmonary presentations are most common and underscore the necessity of careful and timely diagnostic evaluation of pulmonary abnormalities in these patients. Interestingly, the unusual site of rectal involvement described frequently in American series consisting largely of male homosexuals is not observed in an Italian series consisting almost entirely of iv drug users (*43*).

CENTRAL NERVOUS SYSTEM INVOLVEMENT IN ACQUIRED IMMUNODEFICIENCY SYNDROME–NON-HODGKIN'S LYMPHOMA

Involvement of the CNS, usually meningeal, occurs in 10%–20% of AIDS–NHL patients presenting with other extracranial sites of involvement. Such involvement is frequently associated with that of bone marrow and may occur with small noncleaved or diffuse immunoblastic lymphomas (*23, 30–32*). The CNS is also a frequent site of relapse or progression in all AIDS–NHL patients (*25, 76–79*), and this has led to the recommendation that CNS prophylaxis be included in the primary treatment of AIDS–NHL (*24, 25*). Because AIDS patients are subject to multiple intracranial pathologic states, the appearance of which may be difficult to differentiate by computed tomogram or magnetic resonance imaging, early biopsy with cytology, histology, and culture of lesions is recommended (*76*). Magnetic resonance imaging studies with gadolinium as a contrast agent have shown promise for evaluation of this population of patients (*77*).

Primary CNS lymphoma in AIDS patients may account for up to one-fourth of all AIDS–NHL (*30, 76*) and was recognized early as one of the AIDS diagnostic criteria (*20*). These are almost always high-grade B-cell tumors with small noncleaved or large cell immunoblastic lymphomas that display a high degree of multicentricity, particularly at autopsy (*78, 79*). In a series of 20 patients reported by So et al. (*79*), the most common presenting symptoms were confusion, lethargy, and memory loss with other focal neurologic findings being common as well. Abnormal spinal fluid was observed in 82% of these patients, usually with mild pleocytosis, elevated protein, or depressed glucose. Cytologic evaluation showing atypical or malignant cells was positive in only 2 of 8 patients. Although these tumors are radioresponsive, survival has generally been poor, with a mean of 2–5.5 months (*79, 80*). Peripheral nerve involvement

Table 4.—Frequency of unusual anatomic locations of involvement with AIDS-NHL[a]

Site	No. of patients
Lung, pleura	17
Intraoral, Waldeyer's	14
Rectal	9
Skin	7
Soft tissue	6
Salivary glands	4
Cardiac, pericardial	3
Bone	3
Testis	2
Ascites	2
Pleural fluid	2
Kidney	1
Adrenal	1
Bladder	1
Paranasal sinus	1
Conjunctiva	1
Cranial nerves	1

[a] In the following series, all are reported sites *excluding* bone marrow, CNS (parenchymal or meningeal), liver, and gastrointestinal tract (*25, 26, 28, 30–32*).

by direct lymphomatous infiltration that occurs in AIDS–NHL has also been described (*81*).

CLINICAL CHARACTERISTICS OF ACQUIRED IMMUNODEFICIENCY SYNDROME-ASSOCIATED HODGKIN'S DISEASE

The clinical characteristics of AIDS-related Hodgkin's disease parallel those of the AIDS–NHL. In general, patients with AIDS–Hodgkin's disease present with advanced-stage disease, B symptoms (which may be mimicked by opportunistic infections and intestinal malabsorption), unusual anatomic sites of involvement, and greatly shortened survival, compared with those who do not have AIDS. In the series of 13 patients with Hodgkin's disease and AIDS reported by Knowles et al. (*31*), 9 presented with stage IV disease, 11 had B symptoms, and 8, 2, and 1 had bone marrow, liver, and lung involvement, respectively. One stage II patient was treated with radiation therapy and, while in remission, died of an opportunistic infection. The rest received MOPP and ABVD. One patient died on the first day of therapy. Three died of opportunistic infections while in remission. Five patients in partial remission died of infection as well, and 1 died of Hodgkin's disease. Median survival for the group was 14 months. In a separate series reported from Stanford, 14 of 19 patients with AIDS-associated Hodgkin's disease had stage III or IV disease (*45*). Unusual sites included disease of the CNS (3), skin (2), endobronchial (1), and mesenteric involvement (1); 1 patient had the unique presentation of bone marrow involvement in the absence of splenic involvement. Kaposi's sarcoma or opportunistic infection that developed in 42% always followed the diagnosis of Hodgkin's disease. Once these former diagnoses were made, survival was only 1–6 months.

PROGNOSTIC FACTORS IN ACQUIRED IMMUNODEFICIENCY SYNDROME–NON-HODGKIN'S LYMPHOMA

In general, the prognostic factors of significance for AIDS alone dominate those related to lymphoma in the AIDS–NHL. For example, in the series reported by Ziegler et al. (*23*), no correlation was seen between complete response rate and clinical stage or histologic grade. (Low-grade histologic type was associated with lengthened survival.) Poor prognostic factors included preexisting diagnosis of AIDS, primary CNS lymphoma, high- or intermediate-grade histology, and incomplete response to therapy.

The prognostic significance of histologic subtype is inconsistent in the reported literature. Kalter and associates (*28*) characterized their group of 6 patients with large cell lymphoma (1 patient had an immunoblastic sarcoma) as having a high incidence of CNS involvement, opportunistic infections before the diagnosis of lymphoma, profound T-cell dysfunction, poor performance status, and poor response to therapy. In contrast, of 7 patients with diffuse undifferentiated lymphomas, only 1 had CNS disease; all had good performance status, and 5 of 7 achieved complete remissions. In the series of Knowles and co-workers (*31*), the median survivals, which varied according to histopathologic subtype, were 7.5, 5.5, and 2.5 months for large noncleaved, small noncleaved, and immunoblastic plasmacytoid subtypes, respectively. Karnofsky performance status was the most important predictor of response, and survival was not related to CNS involvement, stage, or histologic subtype, although the trend was statistically nonsignificant for the immunoblastic sarcoma histology to be associated with poor prognosis for response to therapy in the study by Gill et al. (*25*).

Another investigation confirming the dominance of prognostic factors related to AIDS alone in AIDS–NHL is the series of 84 patients reported from San Francisco (*32*). The most important predictor of survival, when available, was the total number of CD4-positive lymphocytes; patients with a total CD4 count greater than 100 cells/mm^3 had a median survival of 24 months, whereas those with CD4 counts less than 100/mm^3 had a median survival of 4.1 months. No other factors contributed additional information when CD4 counts were available. In the absence of CD4 counts, no prior AIDS diagnosis was the best predictor of survival. The median survival of a patient without AIDS was 8.3 versus 2.2 months for one with a prior AIDS diagnosis. However, survival from the first AIDS diagnosis was the same for patients whose initial diagnosis was lymphoma, compared with those whose initial diagnosis was an opportunistic infection or other cancer. Less important prognostic factors were Karnofsky performance status (<70%, median survival = 3.8 mo; >70%, median survival = 6.8 mo) and presence of extranodal disease (lymphadenopathy alone, median survival = 12.2 mo; extranodal or extranodal ± nodal disease = 3.4 and 4.2 mo, respectively). In this series, survival was not influenced by the number of extranodal sites, specific site of extranodal disease, or histologic subtype. Thus it would appear that the degree of immune compromise, as measured by CD4 counts, prior diagnosis of AIDS, and performance status are clear prognostic predictors. The definition of the prognostic influence of intermediate- or low-grade histology, histologic subtype, and number and location of extranodal sites awaits further studies in large numbers of homogeneously treated patients with uniform pathologic review.

THERAPY

The therapeutic challenge in the AIDS–NHL is the application of treatment principles useful for NHL in the presence of a second lethal disease (AIDS). The very nature of this second disease characterized by immunosuppression, opportunistic infections, and poor bone marrow function makes such therapy more difficult with increased toxic effect. Most AIDS–NHL patients have been treated with standard combination chemotherapy regimens known to be effective in intermediate-grade NHL (diffuse aggressive lymphomas). The results of this experience have been disappointing, with poor complete response rates in the range of 50% or less, high relapse rates, and high mortality related to infectious complications, compared with the experience of such regimens used in non-AIDS-associated lymphomas. Ziegler et al. (*23*) reported that only 53% of patients achieved a complete response, and at the time of that report more than half of those had relapsed. The median survivals of 5 and 6 months for patients with intermediate- and high-grade lymphomas, respectively, were noted to be 50% and 88% shorter than median survivals in published series for non-AIDS-related NHL. This overall experience has been confirmed in several other studies (*24–27, 29, 32*) with most patients being treated with the following regimens:

Regimen	Reference
CVP	(*82*)
CHOP	(*83*)
COMP	(*84*)
BACOP	(*85*)
M-BACOD	(*86*)
COMLA	(*87*)
ProMACE/MOPP	(*88*)

Although some investigators, as discussed in the Prognostic Factors section (*28, 30, 31*), have attempted to identify subgroups with better response rates to aggressive combination chemotherapy in retrospective analyses of patients treated with various regimens, the overall outlook for therapy of the AIDS–NHL has remained poor. The first prospective treatment trial with a regimen specifically designed for AIDS–NHL patients was reported in 1987 by Gill et al. (*25*). In this trial, AIDS–NHL patients (high-grade B-cell) were accrued onto two sequential phase II studies, the first being M-BACOD (*86*) with 13 patients, and the second, a novel regimen of intensive induction therapy with high-dose cytarabine, vincristine, asparaginase, prednisone, cyclophosphamide, and high-dose methotrexate with leucovorin rescue. Consolidation therapy was delivered as three cycles of CHOP alternating with three cycles of etoposide every 21 days. Prophylaxis of CNS with 2,400 rad to a helmet field was begun on day 90. The first group achieved a complete response rate of 54%; 31% achieved disease-free survival for more than 1 year, and CNS progression occurred in 15%. In contrast, the complete response rate for the second group was 33%, whereas 11% achieved disease-free survival for longer than 1 year, and 67% experienced CNS progression, although the patients in this group had significantly more bone marrow involvement at presentation. Median survival for the first group was 11 versus 6 months for the group treated with the intensive regimen. The important conclusion of this study was that the intensive regimen was associated with marked hematologic dysfunction and increased early death primarily due to an increased rate of opportunistic infection. In addition, the experience with CNS progressive involvement in this study indicated the need for the early incorporation of CNS prophylaxis in the therapy of AIDS–NHL.

In the more recently reported work of Kaplan et al. (*32*), the response rate, survival, and other prognostic variables of a group of 27 AIDS–NHL patients treated with standard chemotherapy [CHOP (*83*), COMP (*84*), M-BACOD (*86*), COMLA (*87*), or ProMACE (*88*)] were compared with 38 patients treated with a novel regimen "COMET-A" for AIDS–NHL. This dose-intensive regimen consisted of 1.4 g cyclophosphamide/m^2 and 2 mg vincristine on day 1, 500 mg methotrexate/m^2 with leucovorin rescue on day 8, and 150 mg etoposide/m^2 followed by 3.0 g cytarabine over 2 hours on day 15. Prophylaxis of CNS consisted of weekly intrathecal methotrexate for the first 4 weeks. Pneumocystis prophylaxis consisted of 4 mg pentamidine/kg given iv monthly. No significant difference was observed in response rate between the two groups (46% standard therapy, 58% COMET-A), but median survival for those receiving the COMET-A regimen was significantly shorter (5.2 mo) than was that for the patients in the standard therapy group (11.3 mo). This difference was observed even in the subset of patients with the best prognostic factors of no prior AIDS diagnosis and good performance score. In addition, these investigators reported shortened survival associated with chemotherapy regimens containing greater than 1 g cyclophosphamide/m^2 compared with those containing less (4.6 vs. 12.7 mo), and this difference remained significant when the COMET-A-treated patients were excluded from analysis (*32*). The experience gained from this and the novel protocol reported by Gill et al. (*25*) suggest that dose-intensive, myelosuppressive regimens containing high-dose cyclophosphamide (as well as possibly high-dose cytarabine) led to shortened survival in AIDS–NHL patients. In addition, the experience derived from these studies would indicate that the CNS prophylaxis should be incorporated early in the treatment of AIDS–NHL and that adequate prophylaxis of pneumocystis infection be included in such regimens as well. Levine and colleagues (*89*) have reported promising initial results for a low-dose chemotherapy regimen with CNS prophylaxis and AZT maintenance for AIDS-related lymphoma. In our institution (Lombardi Cancer Center), we are piloting a low-dose weekly chemotherapy regimen with early prophylaxis of CNS and pneumocystis, and antiretroviral therapy using AZT, the details and results of which will be presented in the future. A combination chemotherapy regimen incorporating CNS prophylaxis and antiretroviral therapy for AIDS–NHL has been presented by investigators in France [in abstract form (*90*)]. Thus a "second generation" of AIDS–NHL protocols is in progress, the results of which we hope will represent an improvement over present therapeutic options.

THE FUTURE

The AIDS-associated lymphomas are perhaps the only group of "preventable" lymphomas, and their existence further underscores the urgency of education and adoption of practices to interrupt the several routes of transmission of HIV. Antiretroviral therapy clearly alters the natural history of AIDS and AIDS-related complex with improved survival, fewer opportunistic infections, and improvement in parameters of immune function (*91, 92*). The effect of the increasingly widespread use of AZT on the incidence and clinical characteristics of AIDS–NHL is unknown. It is possible that the population of HIV-infected individuals will be shifted toward the early lymphoproliferative stage of AIDS, a condition that may be related to the etiology of the AIDS–NHL. On the other hand, it is possible that the AIDS–NHL will occur increasingly in a less immunocompromised clinical setting and allow for more aggressive therapy and possibly better treatment outcome. Significant myelotoxic effects experienced by AIDS patients receiving AZT alone is of concern in integrating this drug into combination chemotherapy for AIDS–NHL. In a study reported by Richman et al. (*93*), 40% of the AIDS patients receiving AZT for 6 months required dose reductions or discontinuation of the drug because of myelosuppression. Therefore, one of the present challenges in AIDS–NHL is integration of AZT into combination therapy with acceptable toxicity, or the use of other effective antiretroviral agents with less myelosuppressive potential.

The various CSF are of obvious interest in the hematologic support of AIDS–NHL patients. Human recombinant GM-CSF clearly results in temporary but significant increases in circulating leukocytes and occasionally lymphocytes when administered to patients with advanced cancer (*94*). In a study of

patients receiving G-CSF in the setting of chemotherapy for urothelial tumors, Gabrilove et al. (*95*) reported a reduction in duration of neutropenia, mucositis, and number of days of antibiotic therapy, with an increased proportion of patients able to receive planned chemotherapy. The interaction of GM-CSF with retrovirally infected lymphocytes is complex, with reports of inhibition of HIV replication and potentiation of tumoricidal capacity of monocytes or macrophages (*96, 97*). Stimulation of HIV replication in peripheral blood monocytes has also been reported but in association with enhancement of the antiviral effect of AZT (*98*). Nonetheless, the administration of GM-CSF to AIDS patients by Groopman et al. (*99*) demonstrated an increase in circulating neutrophils, eosinophils, and monocytes, and in a recently presented abstract, Kaplan et al. (*100*) demonstrated similar results in a group of AIDS–NHL chemotherapy patients treated with GM-CSF.

Prophylaxis of *Pneumocystis carinii* pneumonia is a critically important aspect of treatment of AIDS–NHL. Recent reports of the use of aerosolized pentamidine as prophylaxis of pneumocystis in AIDS patients have suggested efficacy with minimal toxic effects (*101, 102*). These characteristics, particularly the lack of myelosuppression, make aerosolized pentamidine an attractive agent for such treatment in AIDS–NHL. An attempt at immune reconstitution with allogeneic bone marrow transplantation and AZT has also been reported (*103*); further studies in this area will be eagerly awaited.

With these developments and the application of principles learned in the first efforts to treat the AIDS–NHL, rational strategies for new treatment protocols are emerging. Thus we can reasonably hope that future treatment of these diseases can be accomplished with fewer side effects and with improvement in the quality of life for patients with this uniquely challenging group of malignant lymphomas.

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Late Complications of Hodgkin's Disease Management

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ABSTRACT—In the past several decades, Hodgkin's disease has been transformed from a uniformly fatal illness to one that can be treated with the expectation of long-term remission or cure in the majority of patients. Because patients now survive for long periods after curative intervention, various complications have been identified. The spectrum of complications following curative therapy is quite diverse and includes immunologic, cardiovascular, pulmonary, thyroid, and gonadal dysfunction. In addition, second malignant neoplasms in the form of acute leukemia as well as secondary solid tumors have now been documented to occur with increased frequency in patients cured of Hodgkin's disease.—J Natl Cancer Inst Monogr 10:55–60, 1990.

Effective therapy has transformed Hodgkin's disease from a commonly fatal disorder to one that is curable in most patients (*1, 2*). Although most long-term survivors enjoy a normal quality and length of life, late complications related to curative treatment are now well recognized. These result from the direct injury to tissues caused by the use of radiation or chemotherapy used for curative intent, or they result from complications of surgical staging and splenectomy, or from the persistent immunologic deficits caused by the underlying illness or its therapy (*3*). In addition, the disease or therapy-related immunosuppression, coupled with the mutagenic effects of chemotherapy and radiation therapy, likely predisposes these patients to second cancers. The spectrum of these late complications is important for the clinician to recognize, as more and more cured Hodgkin's disease patients become part of everyday medical practice. Although the remainder of this paper will focus on these complications, it is important for the reader to recognize that 40 years ago, the vast majority of patients with Hodgkin's disease died from their primary illness and were never at risk for a second cancer. In some sense, late complications are the price we pay for successful therapy. The next decades should bring changes that make therapy equally or more effective while reducing long-term toxic effects.

CATEGORIES OF COMPLICATIONS

The late complications of Hodgkin's disease can be grouped into several broad categories. These include: immunologic disorders and complications of splenectomy, thyroid dysfunction, cardiovascular and pulmonary dysfunction, infertility, and second cancers.

ABBREVIATIONS: ABVD = doxorubicin–bleomycin–vincristine–dacarbazine; MOPP = mechlorethamine–vincristine–procarbazine–prednisone; ANLL = acute nonlymphocytic leukemia; NCI = National Cancer Institute.

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Immunologic Dysfunction and Complications of Splenectomy

Lymphoid tissues are commonly involved in Hodgkin's disease. Indeed, Reed-Sternberg cells appear to derive from antigen-presenting dendritic cells. At diagnosis, a spectrum of immune dysfunctions may exist that are related to the underlying disease, and no doubt, are also enhanced by splenectomy and subsequent therapy. Certain deficits, such as reduced circulating T lymphocytes, may persist without evidence of recovery during remission (*3*).

In splenectomized patients, the cumulative risk of serious bacterial infection approaches 28% within 6 years (*4–6*). However, 75% of these infections occur during therapy and are related to corticosteroid administration or treatment-induced granulocytopenia, rather than to the splenectomy per se. Other infections are secondary to surgical procedures required for diagnosis, staging, or management. Thus the majority of infections cannot be attributed directly to splenectomy. However, gram-positive and encapsulated gram-negative organisms cause serious infections in splenectomized patients due to defective opsonization and clearance (*7*). The cumulative risk of pneumococcal sepsis within 10 years is under 7% (*8, 9*). Obviously, one can decrease this risk through careful selection of patients for staging laparotomy. However, fulminant sepsis is fatal in 33% of the patients, and prompt institution of antibiotic therapy is essential. Most pneumococcal sepsis occurs in children and young adults (<20 yr old) and rarely occurs in older patients in long-term remission. Although prolonged antibiotic prophylaxis has been shown to decrease the carrier state and infection rate in children with sickle cell anemia (*10*), its success in Hodgkin's disease is poorly established. Although pneumococcal vaccination is less effective in splenectomized and/or treated patients (*11–13*), it has little risk and should be administered before splenectomy and therapy. Unfortunately, vaccinated patients sometimes develop infections with serotypes not included in the vaccine (*14*) or with poorly immunogenic serotypes (*15*).

Reactivation of latent herpes zoster/varicella virus can also complicate Hodgkin's disease (*16*). Approximately 16% of the patients eventually develop zoster, with three-fourths of these presenting within 18 months of therapy (*17*). The risk after combined modality therapy (27%) is greater than either radiation (11%) or chemotherapy (13%) alone. Mortality is low (<0.1%), but the morbidity associated with dissemination is often substantial. Therefore, it is recommended that management of zoster in patients recently treated for Hodgkin's disease should include antiviral therapy (acyclovir), initiated within 72 hours of skin eruption.

Thyroid Dysfunction

A progressive elevation of thyrotropin occurs in 31% to 53% of the patients who are treated with mantle radiation (*18*).

However, clinical hypothyroidism develops in approximately 6% to 25% of the patients and may not become apparent until 4 or 5 years after therapy. Chronic stimulation of residual radiation-damaged thyroid tissue by excessive secretion of thyrotropin can induce thyroid cancer in animal models (*19*). As a result, concern has been expressed about a potential thyroid cancer risk. Although palpable thyroid nodules or diffuse enlargement of the gland develops in 43% of the patients within 10 years (*20*), most of these lesions are benign. Overall, only a slight increase in cancers has been reported. However, the latency for thyroid cancer may be very long, i.e., more than 20 years (*21*), and one should be alert to the late occurrence of such tumors in Hodgkin's disease patients who have received radiation therapy. Chemotherapy does not produce thyroid dysfunction when used alone. The large iodine loads associated with lymphangiography and contrast computed tomography can lead to profound but transient disruptions in thyroid function accompanied by elevated thyrotropin levels (*22*). Clinicians should periodically check these levels. The prompt use of thyroid replacement at the onset of a progressive rise in thyrotropin may minimize the consequences of radiation-related thyroid dysfunction.

Cardiovascular and Pulmonary Dysfunction

Acute radiation pneumonitis and chronic restrictive fibrosis can occur following mantle radiation. Approximately 20% of the patients develop symptoms or chest x-ray changes characteristic of acute radiation pneumonitis approximately 3 months (range, 0 to 60) after treatment (*23*). In most instances these changes are asymptomatic. However, approximately one-quarter of the affected patients receive some therapy, usually with a short tapering course of corticosteroids. Complications from radiation therapy are generally related to the total dose and technique (*24*). Lung blocks reduce pulmonary radiation to 6% of the full midplane dose and will cut the incidence of acute radiation pneumonitis in half. In patients with large mediastinal masses, the use of a "shrinking field" technique will allow adjustment of port size in response to tumor regression. Field size can then be modified to allow further reduction in the extent of normal tissue treated.

The risk of pulmonary toxicity is greater following certain types of combined modality therapy, but is low with chemotherapy alone. In a randomized study, the incidence of pulmonary changes following ABVD plus radiation was 49% compared with 15% after MOPP plus radiation (*25*). Chronic dyspnea on exertion developed in 7% of the patients with abnormal chest x rays following therapy with ABVD plus radiation (*26*). Bleomycin-induced pulmonary fibrosis and "radiation recall" pneumonitis induced by doxorubicin may be responsible for the increased toxicity of ABVD. Depending on the clinical setting, a lung biopsy may be required to exclude opportunistic infection in patients with pneumonitis.

Chronic restrictive fibrosis can develop from 9 to 12 months after radiation or combined modality therapy. It is heralded by chest x-ray findings, restrictive changes in pulmonary function tests, or a reduction in diffusing capacity. The abnormalities usually stabilize within 1 or 2 years and seldom require specific therapy.

Cardiovascular injury can occur after mantle irradiation and is primarily manifested by acute or chronic pericarditis and/or myocarditis. Accelerated coronary artery disease is uncommon but may occur (*27, 28*). Excessive cardiac injury due to the use of a single anterior, or anteriorly weighted, radiation port was once a common and serious problem. However, modern techniques require weighted anterior and posterior ports and subcarinal blocks, and cardiac complications are now uncommon. Nevertheless, subclinical asymptomatic pericardial and myocardial dysfunction is seen in over 50% of the patients when they are studied with chest x ray, echocardiogram, and/or radionuclide ventriculogram (*29, 30*). These changes are generally neither serious nor progressive and rarely require specific intervention.

The incidence of clinically apparent acute pericarditis in large radiation series is approximately 13% (*23*). Without pericardial irradiation, the incidence is 7% and is further reduced to 2.5% with subcarinal blocks. Symptoms usually begin within 5 to 9 months, although occasionally they may not occur for several years. About one-third of the patients require nonsteroidal medication and pericardiectomy is required in 16%. Pericardial effusions develop in 25% to 30% of the patients within 2 years (*31*) or sometimes years later (*32, 33*). The onset of symptoms in patients with chronic constrictive pericarditis is usually gradual. Unexplained dyspnea in a patient previously treated with irradiation may require a right heart catheterization to rule out constrictive pericarditis. If pericardiectomy is required, a broad anterior pericardiectomy provides more effective long-term benefit than does a limited window.

Gonadal Dysfunction

Gonadal dysfunction occurs commonly from curative chemotherapy (*34*) and more frequently in men than in women. Many male patients actually have impaired spermatogenesis prior to receiving any treatment (*35, 36*). This pretreatment azoospermia is more common in patients with advanced disease and "B" symptoms and may limit the effectiveness of frozen sperm banking (*37, 38*). Exposure to small amounts of diagnostic radiation is an additional cause of gonadal dysfunction, and even small amounts of radiation, such as the 8–10 cGy required for accurate staging, may cause temporary oligospermia (*39, 40*).

Combination chemotherapy, especially with alkylating agents, is the major cause of male gonadal dysfunction. Following MOPP, virtually 100% of the patients develop azoospermia. Less than 10% of these men recover over a 10-year period (*34*). Also, ABVD frequently induces azoospermia; however, recovery is nearly complete within 2 years (*41*). Gonadal damage can often be documented by serial semen analyses and base-line luteinizing hormone–releasing hormone-stimulated luteinizing hormone and follicle-stimulating hormone levels. Some decrease in testosterone may occur from Leydig cell damage, and the occasional patient may benefit from testosterone replacement (*42*). Although several techniques for prevention of gonadal injury have been studied, none has proved successful to date. The luteinizing hormone–releasing hormone analogues have not protected patients from gonadal injury, perhaps because of the long time required for effective suppression of spermatogenesis before initiation of chemotherapy (*43*).

Women develop gonadal failure less commonly. The ovaries are more resistant to damage than are the testes, presumably because oocytes are not in a continuous proliferative state.

During staging laparotomy, the ovaries can be moved peripherally to reduce exposure to 10% of the midplane dose, which will preserve function in about 60% of the patients who require total nodal irradiation (*44*). Transient amenorrhea occurs in about half the women treated with chemotherapy (*45*). The incidence of permanent ovarian failure is higher following combined modality therapy with pelvic radiation and is clearly related to age (*46, 47*). Eighty to ninety percent of the women over age 25 will eventually develop premature menopause, even if initially they experience only transient amenorrhea. In contrast, fewer than 30% of the women under 25 experience premature menopause. Monitoring of serial follicle-stimulating hormone levels in premenopausal women may reveal a progressive rise beginning approximately 1 year prior to cessation of ovulation. These women continue to have normal menstruation during that period and may still conceive prior to the onset of permanent amenorrhea and infertility. The symptoms of premature menopause can be corrected with cyclic hormonal replacement therapy, although this does not alter ovulatory status. At present, no proven preventive measures are available. Chapman and Sutcliffe (*48*) suggest that oral contraceptives help prevent loss of oocytes during therapy.

Radiation and chemotherapy have well-documented teratogenic effects, and as a result, physicians have been concerned about risks to offspring. For women who resume menstruation, one does not see reduced conception rates, increased fetal wastage, congenital abnormalities, or subsequent abnormal childhood development (*46, 49–51*). Male patients who recover spermatogenesis have also successfully fathered offspring without an increased risk of abnormalities (*51–53*). Therapy during the first trimester of pregnancy may be associated with fetal abnormalities or spontaneous abortion, and women who require treatment during this period should consider elective abortion (*54*). Chemotherapy during the third trimester has not been associated with adverse fetal effects.

Second Cancers

Two broad patterns of second cancers are now known to occur in cured Hodgkin's disease patients. The first is ANLL which appears to be primarily chemotherapy driven, and the second is secondary solid tumors, which occur later and are primarily due to radiation. In both of these complications, the use of combined modality therapy may increase the incidence. Following irradiation alone (table 1), ANLL is extremely uncommon. Following chemotherapy alone, the risk is increased, however, but not as much as when continued maintenance chemotherapy or combined modality treatments are used. Among the patients treated with MOPP alone at the NCI (*58*) who have been followed for up to 20 years, only one case of ANLL has been seen (actuarial risk of 2% at 10 yr). The risk with nitrosourea-containing regimens or with maintenance chemotherapy is higher (*59, 60, 66, 67*). With chemotherapy that does not contain alkylating agents (i.e., ABVD), the risk appears lower (*61, 62*). In patients treated with combined modality therapy, the risk increases as the radiation port size and dose are increased. Also, ANLL is more likely to occur in patients treated for Hodgkin's disease who are over the age of 40 (*64, 65*).

The risk of acute leukemia does not rise continuously over time. In patients treated at the NCI, the risk of ANLL and/or myelodysplastic syndromes peaked at 4 to 6 years after therapy and then declined. Beyond 11 years, the risk approached that of a normal population. In several large series now published, no patient has developed ANLL and/or myelodysplastic syndromes after being off therapy for 12 years or longer. The pattern of risk resembles that after the atomic bomb exposure in Japan, where survivors had an increased risk of leukemia that peaked after 5 to 7 years and then declined but remained slightly elevated for 20 years (*68*).

Table 1.—Risk of secondary ANLL following therapy of Hodgkin's disease[a]

Therapy category	Relative risk	Cumulative risk at 10 yr, %	References
Radiation alone	Very low	0	(*55–57*)
Induction chemotherapy alone			
MOPP	Low	2–3	(*58*)
Carmustine regimens	Intermediate	3–6	(*59, 60*)
ABVD	Low	?	(*61, 62*)
Combined modality radiation and chemotherapy			(*55–57, 63*)
Limited field radiation therapy plus MOPP	Low	2–3	
Extensive radiation therapy plus MOPP	High	4–8	
Salvage therapy	High	5–15	
Age > 40 yr	Very high	25–40	(*64, 65*)
Maintenance therapy			(*66*)
Any	High	5–10	
Prolonged alkylating agents	Very high	10–30	

[a]Data are summarized from multiple reported series, with representative citations noted. Additional specific data can be found in (*63*).

Not only can treated patients with Hodgkin's disease develop acute leukemia, but they can also develop non-Hodgkin's lymphoma (*69, 70*). Lymphoma generally develops late, usually after 6 years or longer. The cumulative incidence at 10 years is 1.3%–4%. Lymphomas of B-cell origin are more common, and they tend to develop in gastrointestinal or retroperitoneal sites.

Increasingly, we are now seeing the late occurrences of second solid tumors in patients cured of Hodgkin's disease. At least five types of solid tumors are seen with increased frequency. These include bone and soft tissue sarcomas, lung and breast cancer, melanoma and carcinomas of the head and neck. In contrast to leukemia, the solid tumors are largely radiation related. Solid tumors have not been seen with increased frequency in patients treated with chemotherapy alone. Bone and soft tissue sarcomas usually occur within irradiation ports after a latency of 6 to 11 years (*63, 71*). The risk of lung cancer is increased 4.6- to 6.3-fold in Hodgkin's disease patients and is associated with mantle radiation. Although it can occur in nonsmokers (*72*), three-fourths of the patients who develop this complication are smokers. Squamous cell carcinoma of the head and neck has been seen with increased frequency and often in patients without other known risk factors; it has a very long

Table 2.—Cumulative risk of solid tumors as second neoplasms[a]

Investigative group[b]	Stages	No. of patients at risk	No. of tumors	Actuarial risk		Reference
				Percent	Years	
Rochester	I-IV[c]	296	18	8.0	10	(*76*)
SWOG	I-IV	659	11	3.0	7	(*64*)
CALGB	I-IV	798	17	9.4	10	(*66*)
Milan	I-IV	1,329	43	6.7	10	(*77*)
Yale	III-B-IV[c]	172	6	5.8	10	(*78*)
Stanford	I-IV	1,507	46	13.2	15	(*79*)
NCI	I-IV	473	22	7.0	10	(*58*)
NCI	I-IV	192	17	15.0	15	(*80*)

[a]Leukemia, myelodysplastic syndromes, non-Hodgkin's lymphoma, and nonmelanoma skin cancer are excluded in most instances.
[b]SWOG = Southwest Oncology Group; CALGB = Cancer and Acute Leukemia Group B.
[c]Patients treated with chemotherapy alone are not included.

latency, e.g., 12–22 years (*73*). Melanoma is also seen with increased frequency, although the major risk appears to be in patients with the dysplastic nevus syndrome (*74*). The late occurrence of breast cancer has now been documented in results from epidemiologic studies from the Connecticut Tumor Registry and the Surveillance, Epidemiology, and End Results Program of the NCI. It may relate to low-dose scatter from mantle radiation (*75*). In data from eight studies, the cumulative incidence of all solid tumors is 5%–7% at 10 years (table 2). In contrast to ANLL, a steady increase in solid tumor risk occurs after the first 10 years, and eventually the risk exceeds that of acute leukemia.

The cumulative risk of solid tumors in the Stanford series rose to 13.2% at 15 years (*79*). Data from the NCI show a similar pattern, with a risk of approximately 12% at 15 years (*80*).

CONCLUSION

Effective radiation and combination chemotherapy have dramatically improved the long-term survival of patients with Hodgkin's disease. Many patients are cured and will enjoy a normal quality of life and a normal life span. However, various complications related to curative therapy have been identified. Some of the more common problems are associated with few symptoms, whereas other less frequent complications are potentially life-threatening. Improvement in therapeutic techniques has already reduced the incidence of many of these complications and has resulted in enhanced long-term survival. Our goal should now be to maintain or even increase the curability of the disease, while reducing even further, the acute and chronic toxicities associated with cure. To reduce infectious complications and immunologic dysfunction, one should avoid staging laparotomy with splenectomy unless the presence of subdiaphragmatic disease would alter the choice of therapy. One should also administer polyvalent pneumococcal vaccine prior to splenectomy or before the initiation of chemotherapy. To reduce the incidence of myocardial or pericardial injury, one can add subcarinal blocks during mantle irradiation unless the patient has pericardial disease. Patients at high risk for pulmonary or cardiac complications should be treated cautiously with doxorubicin combinations, especially after mediastinal radiation. Clinicians must remain alert to the possible diagnosis of chronic restrictive pericarditis in patients with late-onset unexplained dyspnea.

Knowledge of the potential thyroid complications can allow proper management and avoid serious consequences. One should initiate replacement therapy with thyroxin when a progressive rise in thyrotropin level develops following mantle irradiation. One should also carefully evaluate thyroid nodules to exclude late cancer.

To reduce the risk of pulmonary injury, one should attempt to avoid use of bleomycin and nitrosoureas in combination with radiation. The use of lung blocks or shrinking field techniques, or both, can also limit pulmonary radiation and should always be used unless the patient has parenchymal involvement with Hodgkin's disease.

Gonadal dysfunction in women can be minimized by performing ovariopexy at the time of staging laparotomy in those who are to receive pelvic radiation. One should also monitor follicle-stimulating hormone levels in women in the 25- to 35-year age range in order to provide proper counseling in regard to fertility. In women with premature ovarian failure, one should promptly initiate cyclic estrogen/progesterone replacement.

For males, one should consider frozen sperm banking (if semen analysis is adequate prior to therapy) and attempt to minimize risk by considering induction regimens that do not rely on intense and prolonged use of alkylating agents. One should always properly counsel patients regarding the incidence and duration of azoospermia expected after the chosen therapeutic approach.

The risk of second cancers, either acute leukemia or solid tumors, remains the most ominous of the late complications. One may minimize this risk by

1) using combined modality therapy only in those subsets of Hodgkin's disease patients with proven benefit (i.e., massive mediastinal disease);
2) eliminating maintenance chemotherapy in complete remissions;
3) avoiding intermittent reinduction therapy;
4) selecting effective induction chemotherapy regimens with limited or no use of alkylating agents or procarbazine;
5) carefully defining patients at high risk for radiation-induced solid tumors;
6) insisting that cured Hodgkin's disease patients stop smoking;
7) carefully surveying patients for the dysplastic nevus syndrome; and

8) searching for mechanisms to restore normal immune competence.

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Therapy of Lymphoma Directed at Idiotypes[1]

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ABSTRACT—Anti-idiotype monoclonal antibodies are now available for up to one-third of all patients with B-cell cancer. This is because some antibodies made in the past for individual patients cross-react with the idiotype expressed by other patients' tumor cells. Clinical trials with anti-idiotype antibodies have demonstrated reproducible antitumor effects in patients who have failed conventional treatments. The anti-idiotype antibody treatments are not associated with any significant toxicity and rarely induce immune responses in patients with B-cell lymphoma. They can therefore be used repetitively. Future developments will include the combination of anti-idiotype antibodies with other biologic therapies and with chemotherapy. In addition, one may be able to induce the patient to make an active immune response against the idiotype expressed by his or her tumor cells.—J Natl Cancer Inst Monogr 10:61–68, 1990.

The Ig molecule on the surface of B-cell lymphomas and leukemias can serve as a specific target for therapy (*1*), because B-cell malignant neoplasms arise from cells that have made a genetic commitment to express a unique Ig. For instance, in follicular lymphoma, if the translocation of the bcl-2 gene from chromosome 18 to the unused Ig locus on chromosome 14 is the critical oncogenic event, then it must occur after the cell has already rearranged both its functional Ig heavy- and light-chain genes.

Hopper and Nisonoff (*2*) found that it was possible to generate antibodies that recognize the unique portion of each Ig molecule. These antibodies, called anti-idiotype antibodies, may be produced during normal immune responses to antigens and serve to dampen the proliferation of normal B-cell clones and thus to regulate the immune response (*3–5*).

Anti-idiotype antibodies made in the laboratory against the cell-surface Ig of B-cell tumors can distinguish between the tumor and normal B cells in the host (*6–12*). Inasmuch as the surface Ig molecule of the malignant B cell is not secreted or shed to any large extent into the serum, it would form a stable target for immunologic attack. Because surface Ig normally functions as a receptor for antigens that trigger cell growth, antibodies against that structure might be expected to have profound growth-modulating effects. Indeed, several authors (*13–15*) have provided a number of examples to show that antibodies against the Ig molecule can deliver a direct growth-inhibitory signal to tumor cells in vitro. Others (*16–18*) have shown that anti-idiotype antibodies can have potent therapeutic effects against B-cell lymphomas in animal models. Here we will summarize and update our experience in using anti-idiotype antibodies in the therapy of human B-cell lymphomas.

PRODUCTION OF ANTI-IDIOTYPE ANTIBODIES

The Ig from B-cell tumors can be isolated by several approaches and be used as an immunogen to induce antibodies in a different animal species (*19*). In the approach that we have used, hybrid cells are formed between the human tumor cell that expresses cell-surface Ig and a mouse myeloma cell that grows rapidly in vitro and has the capacity to synthesize and secrete large quantities of Ig (*20–22*). Such hybrid cells, which we termed "rescue hybrids," secrete large quantities of the human tumor-derived Ig molecule (*22*).

Once the Ig is rescued from the tumor cells, it is purified and used to immunize mice. Spleen cells from the mice are fused with a myeloma cell line, and hybridomas are selected that secrete antibody that reacts with the tumor-derived Ig, but not with other Ig.

The monoclonal antibodies are tested for reactivity with the patient's tumor and for absence of reactivity with normal tonsil tissue that contains B cells as well as many other normal cell types. The antibodies are purified and prepared for use in treatment of the patient.

By using these custom-made antibodies we could prove that each human B-cell tumor has a unique idiotype. We demonstrated this by pooling 17 anti-idiotype antibodies and testing this pool for reactivity with over 300 lymphoma specimens obtained from different patients. No evidence of cross-reaction was detected with this pool of "private anti-idiotypes" (*19*). Thus each of these private anti-idiotype antibodies was suitable for use only in the patient against whose lymphoma it was produced.

However, in subsequent work we found that it is possible to select antibodies that detect idiotypic structures that are shared among the Ig receptors of tumors from different patients. As suggested by Stevenson et al. (*23*), such shared anti-idiotypes can be identified by their cross-reactivity with minor components of normal serum Ig. We examined a panel of 199 anti-idiotypes produced against 67 tumors for reactivity with normal human serum Ig. Twenty-four percent of the antibodies have been found to react with minor components of normal serum Ig. Of the 47 antibodies that did react, 21 have been found to cross-react with the tumors isolated from more than 1 patient (*24*). The frequency of reactivity with each of these antibodies with various lymphomas is shown in table 1; the frequency of

ABBREVIATION: Ig = immunoglobulin(s).

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Table 1.—Reactivity of shared anti-idiotypes with lymphomas

Antibody designation[a]	No. positive/No. tested[b]	Percent
S2-33	11/178	6.2
S37-48	7/172	4.1
S30-47	6/169	3.6
C33-13	5/152	3.3
C33-23	5/151	3.3
C49-15	1/41	2.4
C52-22	1/41	2.4
C45-23	1/41	2.4
H48-3	4/172	2.3
C31-145	3/150	2.0
H27-17	3/172	1.7
B4-1	2/148	1.4
B4-11	2/152	1.3
C39-25	1/103	0.9
S66-76	1/103	0.9
H22-10	1/104	0.9
L50-19	1/178	0.6
L50-5	1/168	0.6
H28-48	1/174	0.6
L46-49	1/177	0.6

[a] All antibodies are of the IgG class except for S2-33, which belongs to the IgM class.

[b] Number positive does not include the patient for whom the anti-idiotype was developed.

cross-reactivity ranged from 0.6% to 6.2%. In aggregate, the 21 antibodies reacted with 49 (33%) of 150 B-cell lymphomas tested. As shown in table 2, antibodies against shared idiotypes react with all histopathologic subtypes of lymphoma. Up to a year may be required for one to produce therapeutic quantities of a private anti-idiotype antibody. By contrast, an appropriate shared anti-idiotype antibody can be selected in a matter of days by testing the patient's tumor biopsy (fig. 1) with the panel of antibodies and flow cytometry or immunohistochemical techniques (idiotyping). Although the current panel of antibodies against shared idiotypes reacts with only 33% of the lymphomas tested, new antibodies of this type should expand that percentage in the future.

Table 2.—Lymphomas expressing shared idiotypes according to histologic subtype

Cell subtype	Tumors with shared idiotypes/ No. tested	No. of anti-idiotypes reactive with lymphoma subtype[a]
Follicular, small cleaved	30/110	14
Diffuse, small cleaved	2/3	2
Small, noncleaved	8/13	7
Immunoblastic, large cell	4/10	4
Small cell lymphocytic[b]	4/6	2
Diffuse, large cell	1/8	1
Total	49/150	

[a] Value represents aggregate number of anti-idiotypes reactive with each histologic subtype.

[b] Cell subtype is that of chronic lymphocytic leukemia.

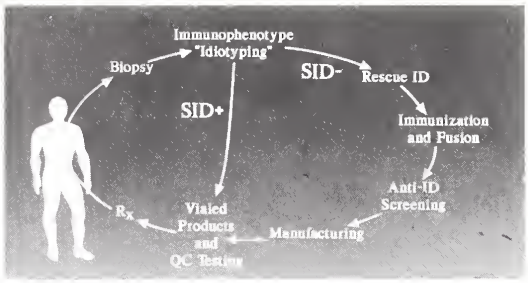

FIGURE 1.—Steps in the production of a private vs. a shared anti-idiotype antibody for therapy. SID = shared idiotype; QC = quality control.

CLINICAL TRIALS

All the results in patients to date have been obtained by our using private anti-idiotype antibodies custom-made for each patient. Three trials of anti-idiotype therapy have been performed, one with anti-idiotype antibody alone (*25, 26*), one with anti-idiotype antibody in combination with interferon-alfa (*27*), and one with anti-idiotype antibody in combination with chlorambucil. The patient selection criteria for these trials are shown in table 3. All the patients had a confirmed diagnosis of lymphoma. Because of the time required to produce a customized antibody, these trials have been limited mostly to patients with an initial diagnosis of follicular (low grade) lymphoma. Patients were required to have an easily accessible site of tumor for biopsy at the time of their entry into the study. The tumor cells had to express an Ig receptor on their surface. This is true for over 90% of patients with follicular lymphoma. During the time of production of the customized antibody, the patients received conventional therapy as appropriate for the stage and activity of their disease. After the antibody was available, assays for idiotype protein were performed on serum samples from the patients. We have found that almost all patients with follicular lymphoma with active disease have some detectable serum idiotype protein. Based on our initial experience, patients were required to have less than 50 μg of serum idiotype protein/mL to be eligible for the later trials. At the time therapy was started, the patients were required to have measurable disease and to have received no other therapy for at least 1 month prior to initiation of the experimental therapy. Finally, the patients were required to have a reasonable performance status. Only a few with follicular lymphoma could meet all these criteria. However, with the advent of shared anti-idiotype antibodies that can be immediately available for patients whose

Table 3.—Clinical trials of anti-idiotype therapy: Patient selection criteria

Diagnosis of lymphoma
Tumor accessible for biopsy
Expression of Ig receptor on surface by tumor cells
Serum idiotype protein level < 50 μg/mL
Measurable disease
Cessation of all previous therapy
Stable clinical performance status

tumors do react, many more patients should be eligible in the future. Most importantly, patients with higher grade lymphomas will be included in the future.

Antibody Administration

We assumed that antibody must reach tumor cells in vivo to have a therapeutic effect. During the course of early studies, we determined that a serum level in excess of 100 μg/mL was associated with detectable antibody in the pleural fluid and in solid tissue biopsy specimens. Thus a peak serum level exceeding 100 μg/mL was chosen for the goal of dosing in these trials. In addition, we attempted to maintain an excess of antibody in the serum and to continue therapy for at least 2 weeks. Generally, patients were infused with monoclonal antibodies iv every 2–3 days with increasing doses as determined by measurements of antibody levels in the serum.

Trial of Anti-idiotype Antibody Alone

Fourteen assessable patients with non-Hodgkin's lymphoma have been treated with anti-idiotype antibodies alone [(*26, 27*); table 4]. Twelve of these patients had relapsed following previous treatment with chemotherapy. Most patients had disease in multiple sites, and 8 had tumor masses greater than 5 cm in diameter. Thirteen of the 14 patients had follicular lymphoma at the time of initial biopsy, but progression to a diffuse lymphoma occurred in the tumors of 3 patients before antibody treatment. The cumulative antibody doses range from 500 mg in patient PK to 15,500 mg in patient KC. In 5 patients, more than one anti-idiotype antibody was used to achieve comprehensive reactivity with the entire tumor cell population. In 16 treatment courses, we observed 2 complete and 6 partial responses. Patient CC had continued tumor regression for more than 27 months and finally achieved a complete response.

Table 4.—Anti-idiotype antibody therapy clinical responses[a]

Patient	Diagnosis	Pretherapy idiotype level, μg/mL	Antibody isotype[b]	Total antibody dose, mg	Immune response vs. mouse Ig	Tumor response	Freedom from progression
PK	FSC, DSC	5.0	IgG2b	400	−	CR	6 yr
CC	FSC	0.01	IgG2a IgG2b IgG1	6,908	−	CR	29 mo
KC	FSC	12.8	IgG2b IgG1	15,500	−	PR	12 mo
JC[c]	DLC	0.8	IgG2a IgG1 IgG1	11,700	−	PR	6 mo
RD	DSC, DLC	0.10	IgG1	1,993	−	PR	5 mo
PE	FSC	14.50	IgG2b	3,183	−	PR	4 mo
CJ	FSC	2.20	IgG1	3,079	+	PR	1 mo
EL[c]	FSC	20.0	IgG1	3,106	−	PR	[d]
CG	FSC	0.01	IgG1	3,173	−	MR	3 mo
BJ	FM	0.02	IgG2b	2,492	+	MR	1 mo
EL[c]	FSC	243.0	IgG1	2,101	−	NR	—
CP	FSC	0.01	IgG1	3,080	+	NR	—
TG	FSC	3.26	IgG2a	1,775	+	NR	—
JC[c]	FSC	49.4	IgG2a IgG1 IgG1	9,630	−	NR	—
EW	FSC	34.0	IgG1 IgG1	9,600	−	NR	—
BL	FSC	3.0	IgG2b IgG1	3,060	−	NR	—

[a] Lymphoma cell types are: FSC = follicular small cleaved cell; DSC = diffuse small cleaved; FM = follicular mixed; DLC = diffuse large. CR = complete response; PR = partial response; MR = mixed response; NR = no response.

[b] All antibodies are mouse monoclonals.

[c] This patient has been treated more than once on this antibody protocol.

[d] This patient was entered on another treatment protocol after receiving antibody therapy, and the duration of the PR could not be determined.

Patients EL and JC were treated twice. In each, the first attempt was unsuccessful because of the patients' high levels of serum idiotype. Both had significant clinical responses once the serum idiotype level was reduced.

When possible, patients with tumors that recurred following anti-idiotype treatment underwent biopsies, and their tumor cells were analyzed for expression of idiotype. In most cases, the relapsing tumors expressed surface Ig. However, in 5 of 10 relapsing tumors analyzed, a predominant population of cells no longer reacted with the therapeutic antibody. The idiotype-negative cells were always derived from the original malignant clone.

Trial of Anti-idiotype Antibody and Interferon

A clinical trial combining anti-idiotype antibodies and recombinant interferon-alfa was performed based on results in an animal model (*28*) and on the independent activities of the anti-idiotype antibodies and interferon in patients with B-cell lymphomas (*29–34*). Anti-idiotype antibody was administered as before, three times per week for 3 to 4 weeks. Recombinant interferon-alfa was given 2 hours before each antibody infusion at a dose of 12×10^6 U/m^2 im. After the first 3 to 4 weeks, the interferon was continued alone at the same dose three times weekly for 8 weeks.

Eleven patients were entered on this protocol; all had follicular lymphoma at the time of initial biopsy, but as in the previous trial, 3 had progressed to intermediate-grade lymphoma during the period that the antibody was being prepared. Nine patients had failed previous therapy with chemotherapy or radiotherapy, or both. Nine patients had disease that was progressing before therapy was initiated. Ten patients had involvement of five or more lymphoid anatomic sites, and patient MW had diffuse involvement of the gastrointestinal tract. Five patients had individual tumor masses of 5 cm or greater.

Table 5 summarizes the treatments of anti-idiotype antibodies and interferon. Two patients, JC and BR, were treated with more than one monoclonal anti-idiotype antibody to achieve comprehensive reactivity with the tumor cell population; BR received two courses of treatment. Individual doses of antibody ranged from 240 to 900 mg. The cumulative antibody dose over the treatment courses in these patients ranged from 2,500 to 8,400 mg, depending on the availability of antibody.

Ten of the 11 patients had tumor regression. In 12 treatment courses, we observed 2 complete, 7 partial, and 2 minor responses. A complete remission was achieved in patient BL and has persisted without any intervening therapy for longer than 32 months. Patient BR had a partial response with progression 3 months following completion of his initial course of therapy. A second cycle of anti-idiotype antibody and interferon-alfa resulted in a complete remission that has persisted for longer than 29 months. In patient BR, the tumor recurrence observed after the first course of antibody therapy was noted to contain idiotype-negative variant cells. This population of idiotype-negative cells was reactive with a second antibody included in the second treatment course.

The combination of anti-idiotype antibody and interferon seemed to produce a higher response rate than that seen with

Table 5.—Anti-idiotype antibody and interferon therapy: Clinical responses[a]

Patient	Diagnosis	Pretherapy idiotype level μg/mL	Antibody isotype[b]	Total antibody dose, mg	Immune response vs. mouse Ig	Tumor response	Freedom from progression, mo
BL	FSC	7.0	IgG1	4,308	−	CR	>32
BR	FSC	1.0	rIgG1 rIgG2a	7,530	−	CR	>29
DT	FSC	12.3	IgG1	6,840	−	PR	13
MW	FM, DLC	19.8	rIgG2a	4,740	−	PR	9
BE	FSC	4.6	IgG1	8,400	−	PR	7
RW	FM, F + DM with DLC[c]	0.1	IgG1	2,930	−	PR	5
BR[d]	FSC	4.0	rIgG1	3,800	−	PR	3
PC	FSC	1.7	IgG1	4,440	−	PR	3
JC	FSC, DLC	1.0	IgG2a IgG1 IgG1	2,500	−	PR	2
RT	FSC	4.7	IgG1	2,880	+	MR	6
KB	FSC	2.5	IgG1	1,680	+	MR	5
RV	FSC	3.5	IgG1	7,440	−	NR	—

[a] *See* table 4 for definitions.
[b] Antibodies are mouse monoclonal antibodies except those with the prefix "r," which are rat monoclonal antibodies.
[c] Diagnosis is follicular and diffuse mixed lymphoma with focal areas of diffuse large cells.
[d] This patient has been treated more than once with this antibody protocol.

Table 6.—Evolution of anti-idiotype treatment approaches

Comments	Anti-idiotype antibody		
	Alone	With interferon	With chlorambucil[a]
Clinical results[b]	2/6/14	2/7/11	1/4/10
Limitations	Serum idiotype Human anti-mouse antibody Antigen-negative cells	Antigen-negative cells	ND
Plan	Combined anti-idiotype with interferon	Combined anti-idiotype with chemotherapy	ND

[a]ND = not done.
[b]Values are expressed as CR/PR/total.

anti-idiotype antibody alone, but the numbers of patients in these trials were small. Interferon was added with the expectation that its anti-lymphoma action would equally affect idiotype-positive and -negative variant cells, yet selection for idiotype-negative variants still occurred.

Trial of Anti-idiotype Antibody and Chlorambucil

In an ongoing clinical trial, we combined anti-idiotype antibodies and the alkylating agent chlorambucil in an attempt to reduce the problem of tumor escape with idiotype-negative variant cells. Because idiotype-negative cells are a small fraction of the pretreatment tumor, their prevalence in escaping tumors following tumor reduction with anti-idiotype therapy indicates that these cells must be proliferating. For this reason, chlorambucil has been added to the treatment regimen at a time when idiotype-negative variant cells are likely to be proliferating. In this regimen, anti-idiotype antibody is given three times per week for 3 weeks. This is followed by a 1-month rest period, and the anti-idiotype antibody treatment is repeated with one course of chlorambucil given at a dose of 16 mg/m^2 for 5 days. All patients entered onto this trial have also failed previous chemotherapy. Of 10 patients entered on this protocol, we have had 1 complete response, 4 partial responses, 2 each minimal and mixed responses, and 1 no response. The response durations have ranged from 3 to more than 23 months. Immunologic characteristics of relapsing tumors are being analyzed, but we do not know if the chlorambucil has reduced the problem of tumor regrowth with idiotype-negative cells. This information will be obtained with longer follow-up and analysis of recurrent tumors. A comparative summary of the three clinical trials is shown in table 6.

RESPONSES OCCURRING IN HIGHER GRADE LYMPHOMAS

All the patients in these clinical trials have had follicular low-grade lymphomas at the time of biopsy for production of anti-idiotype antibody. However, as pointed out above, many patients had undergone histologic conversion to a higher grade histology at the time of initiation of treatment with the anti-idiotype antibody (table 7). One complete and 6 partial responses were seen in a total of 10 such patients. These results indicate that anti-idiotype antibodies have activity in the more aggressive lymphomas as well as the indolent low-grade lymphomas. The high response seen in patients with histologic conversion is impressive, given the poor prognosis associated with this condition (*35*).

Toxic Effects

Toxic effects of these antibody infusions were mild. The most frequent side effects included chills and fever. Transient dyspnea, headache, nausea, emesis, diarrhea, or myalgias were occasionally observed. We alleviated these transient side effects by either reducing the rate or by temporarily discontinuing the antibody infusion. These reactions usually occurred during the first hour of infusion and were not observed after serum idiotype was cleared or excess mouse antibody was achieved. Patients who did not have idiotype protein or malignant cells in the blood at the time of infusion could tolerate antibody infusion rates as high as 200 mg/hour without side effects. Similarly, patients with residual mouse antibody from a prior dose had no adverse effects.

Patients have rarely developed either transient leukopenia or thrombocytopenia lasting less than 48 hours; transient and mild changes in hepatic enzymes including aspartate transferase, alanine aminotransferase, and alkaline phosphatase have occurred occasionally. Elevated lactate dehydrogenase levels have been seen more commonly after anti-idiotype treatment and may be related to tumor cell destruction. During the first week of antibody therapy, several patients developed swelling in one or more lymph node sites that subsided within 2 weeks.

Human Anti-mouse Antibody Response

The development of a human anti-mouse antibody response has been a significant obstacle to successful therapy in most

Table 7.—Anti-idiotype responses in intermediate-grade lymphoma[a]

Patient	Diagnosis[b]	Response	Duration
PK	DSC	CR	6 yr
JC	DLC	PR	6 mo
RD	DLC	PR	6 mo
MW	DLC	PR	9 mo
RW	DM/DLC	PR	5 mo
BE	F/DLC	PR	4 mo
RS	DLC	MR	—
SG	DLC	NR	—
RF	DLC	Stable disease	9 mo +
LV	DLC	PR	3 mo

[a]We observed 1 CR, 6 PR, 1 MR, and 1 NR.
[b]*See* table 4 for definitions.

clinical trials with murine monoclonal antibodies (*36–39*). In our initial report of 9 patients with B-cell lymphoma treated with murine monoclonal anti-idiotype antibodies, 4 immune responses to mouse Ig were seen. Only 2 of the last 25 patients treated with monoclonal anti-idiotype antibodies have developed this type of antibody response. The reduced rate of human anti-mouse antibody formation in patients treated with anti-idiotype antibodies has been attributed to increased purity of the antibody preparations.

CONCLUSIONS

A number of general conclusions can be made from these three trials of anti-idiotype antibody therapy for B-cell malignant neoplasms (table 8). Antitumor effects have been seen in a high proportion of patients. If one considers all degrees of tumor regression, including those that did not reach the classical criteria for partial response of 50% in magnitude at all sites, evidence of tumor response was seen in most of the patients who have been treated. Furthermore, as mentioned above, patients who had converted from follicular to intermediate-grade lymphoma also had a high rate of response. Although the antibodies infused into these lymphoma patients were mouse antibodies, immune responses by the patients against the foreign protein were rare. This may be a special feature of the disease but may also be a consequence of prior chemotherapy or radiotherapy in these patients. The toxicity of this therapy is mild. The major limitation of this therapy has been the difficulty physicians have in producing antibodies in sufficient quantity on a custom basis.

At the present time, the mechanism of antitumor effect of monoclonal anti-idiotype antibodies is unclear, but several intriguing observations suggest that there is more than just a direct antitumor effect to explain the tumor regressions. To begin with, the timing of tumor regression has been noted to occur as early as 1 week after institution of therapy but as late as 4 weeks after the completion of therapy. These regressions can continue for as long as a year after therapy has been administered. That is, objective measurements of tumor masses continue to decrease over a prolonged period. The longest duration of tumor remission to date is 6 years after a single course of antibody therapy. These observations suggest that the antibody infusion has triggered some type of regulatory control by the patient over the growth of the tumor. One such mechanism has been suggested by Lanzavecchia et al. (*40*), who proposed that a T-cell immune response occurs against foreign antigens that have been deposited in the tumor cells and subsequently appear on the cell surface. Such T cells could mediate a long-term effect (*40*). Alternatively, a second-order immune response against the variable region of the tumor idiotype could have been triggered by the therapeutic maneuver. We have no direct evidence for any of these mechanisms at this time, but they are being actively investigated.

Table 8.—Clinical trials of anti-idiotype therapy: General conclusions

Antitumor effects occur in a high proportion of patients.
Higher and lower grade lymphomas are susceptible.
Immune responses against murine antibodies are rare in patients with B-cell lymphoma.
Toxicity is minimal.
The mechanism of tumor regression is unclear: Regression can begin as soon as 1 wk and as late as 4 wk; regression can continue as long as 1 yr; and the longest duration of remission to date is 6 yr.
Relapses can occur in new sites with long-term control of disease in original regressed sites.
Relapsed tumors are derived from the original clone but frequently are composed of cells expressing Ig but lacking reactivity with the anti-idiotype antibody.

One of the most interesting observations to emerge from these trials has been that the individual tumor cells in follicular lymphoma patients have receptors that differ slightly from one to the next in their variable region amino acid sequence (*41*). This appears to be the consequence of ongoing somatic mutation such as that occurring in normal B cells during immune responses (*42*). When anti-idiotype antibodies bind to the receptors on the tumor cells, there may be cells that lack the particular antigenic determinant recognized by the antibody, and these cells may emerge to be the dominant population when relapses occur after initial regression [(*43*); fig. 1]. This indicates that the antibodies had a powerful antitumor effect against the cells to which they did bind. Interestingly, when tumors recur after initial regression, they often recur in anatomic sites that originally were not part of the patients' disease at the time of therapy. Long-term control of the original regressed tumor sites can occur despite progression in new sites. The fact that the receptor is still present, however, implies that it must be important for maintenance and propagation of the tumor, presumably through an interaction with unknown antigens or endogenous anti-idiotype antibodies.

FUTURE CONSIDERATIONS

Because anti-idiotype antibodies now have been demonstrated to be active, the logical extension of this work is for us to combine antibodies with other therapeutic agents. This will be possible because the toxicity of the antibodies is mild. With the availability now of antibodies against shared idiotypes, investigators will be able to test the effectiveness of this therapy in patients with higher grade tumors who could not ordinarily wait the time required to produce customized antibodies. In addition, antibodies can be available for adjuvant trials in patients induced into remission with conventional modalities of therapy. An extension of this therapy will be the use of anti-idiotype antibodies labeled with radioactive agents to deliver directed radiotherapy to tumor sites in vivo (*44*). Such radioactivity has the potential to address variant cells that do not bind the antibody but that are in the vicinity of the cells that do bind the antibodies. Of course, the toxic effects of this approach would be greater, particularly to normal cells in the bone marrow, and autologous bone marrow transplantation support may be required for this type of maneuver. Such trials are currently under way and the early results are promising (*45*).

In the future, we may be able to develop a more active immunotherapy approach based on the induction of an immune response in the patient against his or her tumor idiotype (*46–49*). With the availability of the idiotype protein from the rescue fusions, one can formulate a vaccine that can be administered to the patient to induce such immune responses. Such an active

immune response might be preferable to passive therapy with antibodies, because it would be polyclonal and therefore better able to deal with the problem of tumor heterogeneity. In addition, a T-cell immune response may be directly inducible by this approach. Our experiments in animal model systems indicate that this approach will be feasible (*50*), and trials of idiotype vaccination have already begun.

Because much has been learned recently about the receptor on T lymphocytes, development of a similar approach for the therapy of T-cell lymphomas and leukemias is now possible. Anti-idiotype antibodies can be made against the T-cell receptor in much the same way as are those against the B-cell idiotype (*51–53*). The T-cell receptor is not a secreted protein nor does it undergo somatic mutation (*54*). For these reasons, it is a particularly inviting target for immunologic therapy. This may be true not only for T-cell malignant neoplasms but also for autoimmune disorders in which T cells play a critical role.

Finally, through currently available methodology of genetic engineering, we can now reconstruct the T-cell receptor on killer T cells. One can insert the genes for the variable region of Ig antibody molecules into the receptor of functional killer T cells (*55*). By this approach, one can create T cells with specificity for any tumor against which a monoclonal antibody can bind, such as B-cell lymphomas. These redirected T cells could be used for the therapy of these tumors.

In summary, immunologic approaches to the therapy of lymphoma and leukemia have been devised that take advantage of our knowledge of the unique receptors on the surface of these types of cells. One can imagine a future role for such therapies in combination with other modalities of treatment. The adverse reactions associated with current therapies may be lessened, and their effectiveness may be enhanced by immunologic manipulation.

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Prospects for Interleukin-2 Therapy in Hematologic Malignant Neoplasms[1]

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ABSTRACT—Interleukin-2 (IL-2) is a regulator of diverse functions of the immune system that can induce regressions in some experimental and human tumors. These early findings suggest a potential role for IL-2 in the treatment of certain malignant neoplasms including lymphomas and leukemias. Advanced, rapidly growing tumors are generally not amenable to immunotherapy. Therefore, it is more likely that protocols with IL-2 will be used to prolong remission and prevent relapse in leukemia patients with minimal tumor load. Several approaches are currently being tested in animal experiments and clinical trials. Activation of tumor-reactive lymphocytes (specific or nonspecific) by IL-2 in vivo may eradicate residual leukemia in patients with occult disease. In vitro-propagated autologous or allogeneic leukemia-reactive T cells may be infused with IL-2 to facilitate the tumor destruction. The IL-2 enhances monoclonal antibody-dependent effector systems, such as antibody-dependent cell-mediated cytotoxicity in vivo. Monoclonal antibodies recognizing epitopes on leukemia/lymphoma cells could therefore be used with IL-2 to target nonspecific effectors to destroy tumor cells. Other cytokines appear to potentiate various antitumor activities of IL-2, including cytotoxicity of antigen-specific T lymphocytes or lymphokine-activated killer cells in vitro, and these combined effects may be exploited in clinical trials in which more than one cytokine is used simultaneously or in sequence. Finally, a stepwise completion of clinical protocols testing this immunologic approach in combination with other treatment modalities is necessary. Currently, it is not clear which, if any, of the many hematopoietic tumors are most likely to respond to IL-2 treatment. More information is needed, not only on IL-2 stimulation of host antitumor responses but also on the direct effects of IL-2 and IL-2-induced cytokines on hematopoietic neoplasms.—J Natl Cancer Inst Monogr 10:69–72, 1990.

Despite the advances in chemoradiotherapy and bone marrow transplantation, a considerable number of patients with all types of lymphoma and leukemia die of their disease. Recently, the availability of highly purified recombinant cytokines, such as the interferons, interleukins, TNF, and hematopoietic growth factors, opened conceptually new approaches to treatment of these diseases. Recombinant IFN-α has already become an important tool in the management of hairy cell leukemia, whereas cytokine therapy of other hematologic tumors is still in an experimental stage.

One of the molecules that shows promise is IL-2. This potent regulator of diverse functions of the immune system can induce antitumor responses in many experimental neoplasms including lymphomas. The mechanism of this antitumor activity is currently the subject of intensive investigations. Clinical testing has documented responses in IL-2-treated patients, but these occur only in a minority of cases and most are temporary. Crucial questions regarding the therapeutic use of IL-2 still need to be answered. Researchers conducting laboratory and clinical studies are currently investigating which malignant neoplasms to treat, at what stage, and with what therapeutic schedules.

In this review, we will discuss advances in IL-2 research relevant to its potential use in the therapy of leukemia and lymphoma and summarize future investigations that may be needed to define the role of IL-2, if any, in the treatment of hematologic tumors.

INTERLEUKIN-2 AND ITS RECEPTORS

As a 15,000-molecular-weight glycoprotein of 133 amino acids, IL-2 is synthesized mainly by activated T cells (*1–3*). Originally described as a T-cell growth factor, IL-2 can cause functional changes in T and B lymphocytes, natural killer cells, monocytes, and oligodendrocytes (*4–7*). Incubation of normal lymphocytes with high doses of IL-2 leads to generation of powerful cytotoxic cells (designated LAK cells) that can lyse most autologous and allogeneic tumor cells and cell lines as well as virally transformed cells and certain normal tissues (*8, 9*). The LAK activity is mediated by a heterogeneous population of cells, with most LAK cell precursors being CD3−, CD4−, CD16+, and NKH-1+ (*10*). Unlike the cell lysis by specific CTL, which recognize nominal antigen in association with major histocompatibility complex determinants, the cytotoxic action of LAK cells is not restricted by the major histocompatibility complex (*11*). Also, IL-2 allows the differentiation and proliferation of CTL, including tumor-specific killer T cells in animal models. Playing a central role in the regulation of the immune response, IL-2 can induce the synthesis and release of many other cytokines. In vitro activation of peripheral blood mononuclear cells by IL-2 induces mRNA expression and release of TNF-α, TNF-β, lymphotoxin, IL-6, and IFN-γ (*12, 13*).

The effects of IL-2 are mediated through specific receptors on the cell surface (*4, 14*). High-affinity IL-2 receptors (K_d ~10 p*M*) are formed by the association of two subunits, the Tac-subunit (mol wt = 55,000) recognized by the CD25 (anti-Tac)

ABBREVIATIONS: IL-2 = interleukin-2; LAK = lymphokine-activated killer; CTL = cytotoxic T lymphocytes; TNF = tumor necrosis factor; TIL = tumor-infiltrating lymphocytes; IFN = interferon; K_d = dissociation constant.

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monoclonal antibody and the p75 subunit (mol wt = 75,000). The p55 or p75 subunit alone forms IL-2 receptors with low (K_d ~10 n*M*) or intermediate affinity (K_d ~100 p*M*), respectively (*15*). Both high- and intermediate-affinity receptors are involved in IL-2 signaling. The high-affinity receptor is expressed on activated T and B cells mediating a number of functional changes. The p75 molecule alone is expressed on large granular lymphocytes and mediates the induction of LAK activity (*16*). The expression of IL-2 receptors is not limited to normal lymphocytes. Cells from most acute leukemias (including T-, common, and non-lymphoid leukemias) as well as T-lymphoblastic lymphomas and peripheral T-cell lymphomas express the p75 usually without the p55 IL-2 receptor molecules (*17*). Chronic lymphocytic leukemia, hairy cell leukemia, and other differentiated B-cell neoplasms often express both p55 and p75 subunits (*17*). The precise functional significance of these IL-2 receptors is unclear. In some cases, IL-2 induces proliferation and immunoglobulin secretion in leukemia B cells and LAK cell-like cytotoxicity in neoplastic T cells (*18–20*). It remains speculative whether this differentiating activity of IL-2 could be useful in the treatment of leukemia.

INTERLEUKIN-2 AND LYMPHOKINE-ACTIVATED KILLER CELL THERAPY

Early preclinical studies with IL-2, with or without infusions of LAK cells activated in vitro with IL-2, have demonstrated striking antitumor effects in mice bearing immunogenic and nonimmunogenic tumors. With the immunogenic tumors, T cells presumably recognizing tumor-associated antigens were primarily responsible for this in vivo response, whereas non-T cells (presumably mediating the major histocompatibility complex-unrestricted LAK response) were principally involved in the destruction of the nonimmunogenic tumors (*21, 22*). Interestingly, a biphasic (low dose and high dose) therapeutic optimum was observed in treating mice bearing an experimental transplantable lymphoma with intraperitoneal IL-2 (*23*). The low-dose therapeutic activity was not observed in nude mice, which suggests that it was associated with a T-cell-mediated effect.

The initial clinical trials documenting antitumor effects in humans used protocols consisting of high-dose IL-2 and in vitro-activated autologous LAK cells (*24*). Since then, several groups (*25, 26*) have confirmed that different regimens in which IL-2 was used induced measurable regressions in approximately 10%–30% of the patients with melanoma and renal cell carcinoma. Similar response rates have also been observed in smaller groups of patients with non-Hodgkin's lymphoma and colorectal carcinoma (*24, 27*). Only occasional responses have been noted in patients with other malignant neoplasms. Because the first trials demonstrated a significant dose-related toxicity, several researchers attempted to modify the IL-2 dose and schedule (*25, 26*).

The search for regimens with enhanced antitumor activity and tolerable toxicity has been hampered by the lack of understanding of the IL-2-induced antitumor effects in humans. Preclinical and in vitro data suggest several possible mechanisms. It is conceivable that their relative importance may be different in different tumors. The direct tumor lysis by LAK cells can be demonstrated in vitro and in some animal models. However, LAK activity induced in the peripheral blood lymphocytes of IL-2-treated patients does not necessarily correlate with tumor response. This may be due to other variables involved, such as tumor size and susceptibility to LAK cells, heterogeneity of tumor cell and LAK cell populations, LAK cell mobility, vascular effects, etc. Therefore, it is not surprising that laboratory–clinical correlates are difficult for one to identify (*27*).

A second postulated mechanism of IL-2 therapy is the activation and expansion of tumor-specific T lymphocytes that are detectable in some human tumors (*28*). This is supported by data indicating that IL-2-activated TIL from some patients with melanoma can show a selective cytotoxic activity against the tumor from which they were isolated (*28*). Yet another important component of the anticancer effects of IL-2 may be mediated by the secondary cytokines released from stimulated cells. These molecules can exert immunomodulatory and antitumor activities that may be clinically important. The expression of mRNA for TNF-α and IL-6 can be induced by IL-2 therapy in patients' peripheral blood lymphocytes, and increased levels of TNF-α and IFN-γ have been found in their plasma (*12, 13*). Many of the observed side effects that cannot be explained by direct activities of IL-2 or direct cytolysis of normal tissue by LAK cells have been ascribed to toxicity of secondary cytokines (*12, 13*). The in vivo interactions between these molecules and IL-2 are probably complex. In vitro IFN-γ regulates growth and differentiation of CTL and LAK cells (*29, 30*). It can also change the ability of leukemia and lymphoma cell lines to be lysed by LAK cells (*31*) and TIL (*32*). The diverse effects of TNF-α include direct cytotoxic and cytostatic activities on certain tumor cells and the synergy with IL-2 in the generation of LAK cells (*33*). Many other synergistic and antagonistic activities within the cytokine network may be important for the final outcome of IL-2 therapy.

Although IL-2 does not appear to exert any direct cytotoxic or cytostatic effect on melanoma, renal cell carcinoma, or other carcinomas, many hematopoietic tumors carry IL-2 receptor molecules on their surface (*see* above). In some of the more "mature" lymphoid tumors, IL-2 induces differentiation and/or proliferation in vitro, whereas cells from acute leukemias appear to be unresponsive to IL-2 (*17, 18*). Tumor cell growth due to IL-2 therapy has not been documented, but we (*34*) have observed persistent changes in the phenotype of circulating lymphoma cells from some patients after treatment with this cytokine. Currently, it is difficult for anyone to predict if malignant lymphoid cells might be induced to differentiate or proliferate upon stimulation by IL-2 treatment. Given the variability of these tumors and the lack of satisfactory animal models, some kind of in vitro screening may be useful prior to immunotherapy.

INTERLEUKIN-2 THERAPY IN HEMATOPOIETIC TUMORS

The iv administration of relatively toxic doses of IL-2, with or without LAK cells, induced regressions of advanced non-Hodgkin's lymphoma in a minority of treated patients (*24, 35*). As was true in other tumors, these responses were not durable. Therefore, it seems unlikely that IL-2 as a single agent would play a major role in the therapy of advanced progressive lymphoma. Similarly, patients with rapidly proliferating acute leukemia would be unlikely to benefit from IL-2 alone. Nevertheless, its usefulness in minimal tumor load has been contem-

plated by several investigators (*26, 36, 37*). We know LAK cells that lyse autologous and allogeneic leukemia cells can be generated from patients in remission (*36*). Treatment protocols are being investigated for use in this patient group. Low doses of IL-2 administered to outpatients by constant iv infusion, or sc over many weeks can cause several-fold enhancement of LAK activity in peripheral blood lymphocytes and are generally well tolerated (Sosman JA, Hank JA, Moore KH, et al; Stein RC, Malkovska V, Gordon S, et al: Submitted for publication). The antitumor activity of such a regimen could be tested in an outpatient setting.

The idea of integrating IL-2 therapy into autologous bone marrow transplantation protocols has also been suggested (*38*). In such a setting, the tumor load is minimized, whereas the immunocompetent cells are preserved and infused with the marrow autograft following chemoradiotherapy. Gottlieb et al. (*39*) have demonstrated that IL-2 infusions can be safely administered to patients recovering from chemotherapy or autologous bone marrow transplantation for acute myeloid leukemia and multiple myeloma. In these patients, IL-2 did not significantly suppress the recovering hematopoiesis but induced LAK cells cytotoxic to clonogenic leukemia cells in vitro. Interestingly, Reittie and co-workers (*40*) suggested that IFN-γ-secreting activated killer cells with the capacity to lyse natural killer-resistant targets are normally generated 4–6 weeks after autologous bone marrow transplantation but do not appear after treatment with chemotherapy. The authors speculated that these cytokine-producing cytotoxic cells may be responsible for the lower risk of relapse after autologous bone marrow transplantation compared with chemotherapy alone. Investigators will find it interesting if the potentiation of this autologous "graft-versus-leukemia" activity with IL-2 leads to prolonged remissions.

Following allogeneic bone marrow transplantation, a well-described graft-versus-leukemia (*41, 42*) effect appears to prevent some leukemia recurrences. This effect may be mediated by IL-2 released endogenously as part of the allorecognition response of donor T cells against host tissues. The released IL-2 may activate LAK effector mechanisms and induce the secretion of other cytokines. Alternatively, donor T cells may be recognizing alloantigens (*43*) or leukemia-associated antigens (*44*) on the host leukemia cells and induce specific T-cell-mediated destruction. If the latter is the case, activation, selection, and propagation of such T cells in vitro may enable their infusion (with IL-2 infusion to prolong their in vivo effects) into patients with minimal leukemia burdens, as has been successful in mice (*22*). Propagation of such human clones, with apparent selective recognition of allogeneic leukemia cells, has now been demonstrated (*44*). Some autologous leukemia-reactive lines have also been propagated (*45*).

It has been well-established that other cytokines can potentiate various IL-2-dependent functions, including CTL and LAK-cell cytotoxicity. Many investigators are currently studying whether a better therapeutic index could be achieved by combining IL-2 with other immunomodulators, as has been shown in animal models (*29*).

The therapeutic effect of monoclonal antibodies in lymphoid tumors may depend on the recruitment of host effector systems inducing antibody-dependent cell-mediated cytotoxicity. Using a model in which lymphoma cells are transplanted into mice, researchers (*46, 47*) have demonstrated that IL-2 enhances monoclonal antibody-dependent effector systems in vivo. We have similarly documented a twofold-to-tenfold increase in in vitro antibody-dependent cell-mediated cytotoxicity when the effector cells tested are obtained from patients after 1 month of IL-2 treatment (Hank JA, Robinson RR, Surfus J, et al: Submitted for publication). Such results provide a rationale for using antileukemic monoclonal antibodies with IL-2 in clinical trials.

More information is needed on the direct functional effects of IL-2 and IL-2-induced cytokines on various leukemia and lymphoma cell populations. Specific receptors for these molecules have already been demonstrated on hematopoietic tumors. Studies of the signaling through these pathways may indicate new ways of influencing tumor cell growth and differentiation with cytokines or their analogues.

It is not clear which, if any, of the many hematopoietic neoplasms are most likely to respond to IL-2 treatment. The final therapeutic outcome may depend on both the stimulation of host antitumor responses and direct effects on hematopoietic tumor cells.

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Hematopoietic Growth Factors: Biology and Clinical Application[1]

Janice L. Gabrilove *and* Ann Jakubowski[2]

ABSTRACT—A number of recently identified cytokines have been implicated in the development of blood cells and their functional activation. These include granulocyte colony-stimulating factor (G-CSF), granulocyte/macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), and interleukins-1, -3, and -6. The two that have been studied most extensively clinically are G- and GM-CSF. Biologic differences between these two agents have important implications for their use in particular clinical settings. Whereas G-CSF for the most part has demonstrated lineage specificity in stimulating production of neutrophil granulocytes, GM-CSF stimulates production of all types of granulocytes and is a potent activator of monocytes and macrophages as well. In addition, GM-CSF has been found to induce other cytokines, such as tumor necrosis factor and interleukin-1. Functional differences such as influencing neutrophil migration have also been noted; GM-CSF can be a potent inhibitor of neutrophil migration. The interaction of these and other cytokines in inflammation and injury is the determinant of the ultimate clinical outcome. Potential clinical applications for these growth factors are discussed, and specific clinical studies during which recombinant human G-CSF was used are reviewed.—J Natl Cancer Inst Monogr 10:73–77, 1990

Blood cell development is regulated by various hematopoietic growth factors that mediate the growth, maturation, and activation of hematopoietic cell elements. Several of these factors have been isolated and are now being produced by recombinant DNA techniques in quantities sufficient for study and clinical use.

At least seven major hematopoietic growth factors are involved in the development and functional activation of mature blood cell elements (*1*). The first three are IL-1, IL-3 (also known as multicolony-stimulating factor), and IL-6. These appear to be important for maintaining the survival, proliferation, and cyclic activation of immature, potential stem cells. In particular, IL-1 also has the capacity to enhance the expression of receptors for more lineage-specific growth factors, such as M-CSF, GM-CSF, and G-CSF.

A fourth hematopoietic growth factor is GM-CSF, which stimulates not only neutrophil granulocytes but all granulocytes, and eosinophil granulocytes in particular. At least in vitro, GM-CSF also has the capacity to support the growth of erythroid colonies in the presence of erythropoietin; however, this activity has not been substantiated in vivo.

Until recently, G-CSF was thought to be lineage specific because it promotes the proliferation, maturation, and functional integrity of mature neutrophil granulocytes. New data, however, reported by McNiece and his colleagues (*2*), suggest that G-CSF can also act in synergy with IL-3 to support other lineages, in particular, megakaryocytes, leading to platelet production. Other current data reported by the same group suggest that G-CSF can also promote pre-B-cell activation and growth.

The sixth factor, and last of the four classic CSF, is M-CSF, which appears to be specific for the proliferation, maturation, and functional activation of mature monocytes/macrophages.

The last of the seven major hematopoietic growth factors is erythropoietin, which acts on the blast-forming unit-erythroid and then through the pathway of the colony-forming unit-erythroid promotes the maturation of red blood cells.

CHARACTERISTICS OF THE COLONY-STIMULATING FACTORS

Molecular and Biochemical

As has been noted (*1*), the genes for IL-3, GM-CSF, and M-CSF are located in the same region of chromosome 5, i.e., on the q arm. This region is deleted in the 5q− syndrome, which is characterized by anemia and which can progress to acute myeloid leukemia. This deletion is also the most common chromosomal abnormality seen in acute myeloid leukemia secondary to prior radiation exposure. Because there is a "normal" residual chromosome 5, the intriguing question arises as to whether this deletion unmasks other abnormal genes on the "normal" chromosome 5, or whether a deletion of an important suppressor gene may be closely linked to these hematopoietic regulators. In contrast to these two genes, the gene for G-CSF is located on the q arm of chromosome 17, in a region that contains other genes involved in neutrophil granulocyte development.

Although IL-3 and GM-CSF are products of T cells and therefore are true lymphokines, G-CSF is only known to be produced by monocytes, fibroblasts, and endothelial and epithelial cells. This difference in cell source may have clinical significance, if one considers the leukocyte response in different diseases. For example, in autoimmune diseases in which T-cell activation occurs, one might suspect that the eosinophilia observed was partly due to the production of GM-CSF or similar molecules. In bacterial sepsis, on the other hand, endotoxin has the capacity to stimulate monocytes to produce G-CSF, which gives rise to the pronounced neutrophil response characteristic of this type of infection.

ABBREVIATIONS: IL = interleukin; M = macrophage; G = granulocyte; CSF = colony-stimulating factors; M-VAC = methotrexate–vinblastine–doxorubicin–cyclophosphamide; rh = recombinant human; ANC = absolute neutrophil count(s).

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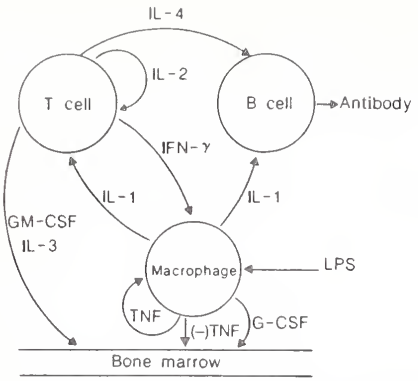

FIGURE 1.—Network of cytokine interaction and positive and negative feedback. IFN-γ = interferon gamma; LPS = lipopolysaccharide; TNF = tumor necrosis factor. Adapted with permission of the publisher (*9*).

Granulocyte and Granulocyte/Macrophage Colony-stimulating Factors: Differences and Similarities

Because G-CSF and GM-CSF are likely to be useful in numerous related clinical settings, we need to consider some of their biologic differences. These may have important implications for our considering the pathogenesis of some diseases and may affect the comparative clinical usefulness of the two factors in these conditions.

The G-CSF specifically stimulates neutrophil granulocytes, whereas GM-CSF is a "pan-stimulator" of all granulocytes. For example, to increase levels of eosinophil effector cells to treat a parasitic infection, one could use GM-CSF, a potent activator of monocytes and macrophages, instead of G-CSF, which is not. This activation includes induction of other cytokines when GM-CSF is administered, including tumor necrosis factor and IL-1. This particular difference may be the reason for the various side effects observed in patients treated with GM-CSF, compared with G-CSF. At the same time, this difference in biologic effects might influence the therapeutic approach; GM-CSF rather than G-CSF might be indicated if enhanced monocyte/macrophage activation was a desired end point.

Whereas GM-CSF is a potent inhibitor of neutrophil granulocyte migration, G-CSF enhances neutrophil migration. As yet, GM-CSF has rarely been detected in serum, and therefore it is thought to be produced primarily locally within the bone marrow or at peripheral sites. However, G-CSF has been detected in serum in patients with certain conditions (*3*), such as bacterial sepsis (*3, 4*). For example, during injury, GM-CSF might be produced locally, functioning as a chemoattractant and inhibiting migration of inflammatory cells away from the site of inflammation. In contrast, G-CSF might enhance the migration of inflammatory cells toward the site. This may be one way in which these two hematopoietic growth factors work in concert in augmenting host defense.

The intricate network of effects and pathways by which these molecules interact in the setting of infection or injury is presented in schematic form in figure 1 (*5*). In bacterial sepsis, for example, exposure to endotoxin will stimulate monocytes/macrophages to produce G-CSF, which then stimulates the bone marrow to produce and release neutrophil granulocytes. Macrophages are also stimulated to make IL-1, which in turn stimulates T cells to produce GM-CSF and IL-3, thereby amplifying myelopoiesis within the bone marrow and further augmenting the leukocyte response.

Table 1.—Potential ramifications of treatment with CSF[a]

Ramification	Potential result (benefit)
Reduction of cancer treatment morbidity	Decreased myelosuppression, with decreased incidence of febrile neutropenia
	Decreased mucositis
Improvement in survival	Dose and schedule
	Dose intensification/ bone marrow transplantation
Alteration in the definition of maximum tolerated dose	New drugs
	Previously tested drugs (myelosuppression, dose-limiting toxicity)
Recruitment of malignant cells into S phase	Enhanced susceptibility of malignant cells to killing by cycle-specific drugs
Use as adjunct to serotherapy with monoclonal antibodies	
Use as agent to prime donors for bone marrow transplantation	
Differentiation induction/maturation therapy	

[a]Table is reprinted with the permission of the publisher (*6*).

POTENTIAL CLINICAL APPLICATIONS

The potential clinical applications of these two hematopoietic growth factors (G- and GM-CSF) are set forth in table 1. Based on the studies to date, hematopoietic growth factors are likely to reduce the morbidity associated with cancer therapy and cancer-related myelosuppression by decreasing the incidence of febrile neutropenia and oral complications. To this end, our group has explored the use of rhG-CSF in the treatment of iatrogenic and disease-related neutropenia.

Our first investigation of rhG-CSF to correct or accelerate recovery from chemotherapy-induced neutropenia was performed in patients with transitional cell carcinoma of the urothelium, who were receiving M-VAC as chemotherapy, and was recently summarized (*7*). The clinical trial was divided into three parts. The phase I portion was designed so we could obtain pharmacokinetic and safety data as well as evaluate the growth factor's biologic effect in vivo on normal hematopoiesis (*8*). In the phase II part, we evaluated the efficacy of various doses of rhG-CSF to abrogate myelosuppression following administration of the M-VAC regimen (*9*). In the third part of the study, which was a variation of the phase II, patients received rhG-CSF only with their first *or* second cycle of chemotherapy. We conducted this "extended" phase II to determine if the favorable results observed in the initial phase II had been biased by the prechemotherapy course of rhG-CSF, or if the cumulative effect of the chemotherapy had influenced the results against the second cycle that had been administered without rhG-CSF.

A second area of study in which rhG-CSF was used involved the treatment of chronic neutropenia in adult (*10*) and pediatric patients (*11*). The pilot studies were based on the work of Drs. Malcolm Moore and Karl Welte, who found that the bone marrow from patients with these disorders produced neutrophil granulocytes when cultured in the presence of rhG-CSF.

PATIENTS, METHODS, AND MATERIALS

Forty patients with transitional cell carcinoma of the urothelium were enrolled in the study during which we evaluated rhG-CSF in the setting of chemotherapy-induced neutropenia. In the phase I study, reported in part (*8*), 25 patients were treated with rhG-CSF for up to 6 days prior to chemotherapy with 1, 3, 10, 30, 60, or 100 μg rhG-CSF $\cdot$ kg^{-1} $\cdot$ day^{-1} that was given by a 30- to 40-minute infusion. At least 3 patients were entered at each dose level. Eligibility to participate in the phase II study (*9*) depended on the patient's having a greater than twofold increase in the averaged ANC on days 5 and 6 of the phase I course of treatment. Before beginning the chemotherapy, a patient who was considered eligible was given a 4- to 7-day "wash-out" period following the phase I part of the study; this period allowed peripheral blood cell counts to return to normal and avoided overlap with cycle activation of progenitors. The chemotherapy consisted of methotrexate on day 0 and vinblastine, daunorubicin, and cisplatin on day 1. Treatment with rhG-CSF in the phase II study began on day 4 and was continued for 8 days at doses of 3–100 μg $\cdot$ kg^{-1} $\cdot$ day^{-1}. Twenty-one patients participated in the initial phase II and were monitored for incidence and duration of neutropenia, incidence of nadir fever and duration of antibiotic therapy, incidence and severity of mucositis, and ability to receive day 14 chemotherapy on schedule. Six patients who demonstrated clinical benefit during their cycle with rhG-CSF, compared with their cycle without the factor, were also permitted to continue receiving the growth factor during subsequent cycles of M-VAC at their request.

Fifteen additional patients (12 treated and 3 untreated controls) were monitored during their first two cycles of M-VAC. In this extended phase II study, patients were treated with 10 or 30 μg rhG-CSF $\cdot$ kg^{-1} $\cdot$ day^{-1} only during their first *or* second cycle of chemotherapy. Incidence of mucositis, requirement for antibiotics, and eligibility for day 14 chemotherapy were again monitored.

The most pronounced biologic effect of rhG-CSF observed during the phase I portion of the study was a dose-dependent 2- to 12-fold increase in white blood cell counts and ANC associated with an increase in band forms in the peripheral blood and morphologic changes that included Döhle bodies and toxic granulation. In addition, the monocyte counts increased as much as 10-fold at the higher dose levels, and there was the suggestion of a decrease in the platelet count during the few days following discontinuation of rhG-CSF treatment only at the 60- and 100-μg $\cdot$ kg^{-1} $\cdot$ day^{-1} levels. Bone marrow cellularity increased, again with an increase in band forms in peripheral blood, and granulocyte/macrophage colony-forming units were detected in peripheral blood at the end of the 6 days of treatment.

The changes in values in the chemical profiles listed in table 2 included an increase in leukocyte alkaline phosphatase, uric acid, lactate dehydrogenase, and alkaline phosphatase. Also at the 100-μg/kg level, a mild increase occurred in the liver enzymes aspartate aminotransferase and gamma-glutamyl transpeptidase. As has been reported for GM-CSF, cholesterol levels for patients at the 60- and 100-μg/kg levels demonstrated a decrease over the treatment period (*7*).

Table 2.—Changes in reference values of chemical profiles observed during phase I study with rhG-CSF

Test	Change
Leukocyte alkaline phosphatase (score)	Increased
Lactate dehydrogenase	Increased
Alkaline phosphatase	Increased
White blood cell count and ANC	Increased (2- to 12-fold)
Döhle bodies	Increased
Toxic granulation	Increased
Polylobe count	Decreased
Band forms in peripheral blood	Increased
Bone marrow myeloblasts (100-μg rhG-CSF level)	Increased
Granulocyte/macrophage colony-forming units in peripheral blood	Increased

In the phase II portion, the changes included an increase in white blood cell counts and ANC as well as the leukocyte alkaline phosphatase scores. Increases in uric acid, alkaline phosphatase, and lactate dehydrogenase levels were noted only at the 100-μg $\cdot$ kg^{-1} $\cdot$ day^{-1} dose. The changes in reference values in both parts of the study usually returned to base line within 1–2 weeks after treatment was discontinued (*7*).

The results of the initial phase II part of the study, including patients at the 100-μg/kg level, which compared hematopoietic and oral side effects in cycle 1 of chemotherapy with growth factor to cycle 2 of chemotherapy without growth factor, are as follows: fewer days of nadir blood counts (3 vs. 42), fewer days of neutropenic fever requiring antibiotics; greater percentage of patients eligible for scheduled day 14 chemotherapy (100% vs. 40%), and less mucositis [only 3 with grade II versus 3 (II), 5 (III), 1 (IV)].

Although the number of patients in each group is small, the results (shown in table 3) of the 12 patients who were treated in the extended part of the phase II study generally favored the cycle during which rhG-CSF was given [cycle (+)]. These findings supported the results of the initial portion of the phase II study.

The predominant side effect observed in this study group that was clearly related to treatment was bone and/or muscle pain. In addition, the systemic side effects observed over the entire group of 40 patients that may have been related to rhG-CSF included occasional headache, nausea, flushing, and chest discomfort. Hypotension occurred in a patient who received 60 μg rhG-CSF $\cdot$ kg^{-1} $\cdot$ day^{-1} during the third cycle of chemotherapy. Near syncope was observed in a patient who received the 100-μg dose and who continued treatment without complication.

To be eligible for the chronic neutropenia study, patients were required to have a history of at least three clinically significant infections that necessitated antibiotic therapy, or one life-threatening infection during the year prior to their entering the study. Base-line studies included at least three complete blood counts that demonstrated a neutrophil count of less than

Table 3.—Results of extended phase II study

Patient reaction	10 μg rhG-CSF				30 μg rhG-CSF			
	3 patients		4 patients		2 patients		3 patients	
	Cycle 1 (+)	Cycle 2 (−)	Cycle 1 (−)	Cycle 2 (+)	Cycle 1 (+)	Cycle 2 (−)	Cycle 1 (−)	Cycle 2 (+)
Mucositis	0/3	1/3 (III)	2/4 (III)	2/4 (I)	1/2 (II)	1/2 (II)	1/2 (I)	1/2 (I)
Need for antibiotics	0/3	0/3	2/4[a]	0/4	0/2	0/2	1/3[b]	0/3
Eligible for day 14 chemotherapy	3/3	3/3	1/3[c]	4/4	2/2	1/2	1/3	3/3

[a]One patient received antibiotics iv for 9 days, and another for 7 days.
[b]One patient received antibiotics orally for 10 days.
[c]One patient was not eligible for scheduled day 14 chemotherapy.

500/μL (for patients with cyclic neutropenia, it was required that they have 5 consecutive days per cycle with a neutrophil count of <500/μL), within the 6 months prior to entry in the study. Patients were randomly assigned to either 4 months of observation first before being crossed over to treatment with rhG-CSF or to begin therapy immediately. The rhG-CSF is given sc daily at doses of 1–5 $\mu g \cdot kg^{-1} \cdot day^{-1}$.

The first adult patient, whose course has been described (*10*), was a 70-year-old woman who presented with a diagnosis of idiopathic neutropenia. She gave a 25- to 30-year history of progressively worsening neutrophil counts, an increasing incidence of infections requiring hospitalization, and aphthous ulcers. The patient's bone marrow was markedly hypocellular and almost devoid of myeloid precursors at the time she began sc rhG-CSF therapy. After 8 weeks of treatment, cellularity was estimated at 60%. Figure 2A illustrates the ANC obtained during the first 5 months of therapy. By the 14th day of treatment (approximately day 30) the ANC had reached 2,500 cells/μL. Following a dose adjustment period, the dramatic oscillating pattern (which was observed initially) dampened, and the patient maintained both a normal cellular bone marrow and ANC after more than 1.5 years on therapy. She has had no significant infections since beginning treatment. Neutrophil function studies, assessed in vitro by phagocytosis of zymosan particles, nitroblue tetrazolium reduction, and in vivo by the leukocyte migration test, have all been normal.

The response of an adult patient with variant cyclic neutropenia appears in figure 2B. The ANC of this patient, although demonstrating a cyclic pattern, rarely reached the 1,000-cells/μL mark. At 6 months into the study, he continued to cycle over approximately 24-day periods, but at a level significantly above 1,000 cells/μL. He also has had no documented bacterial infections since entering the study.

Bonilla et al. (*11*) have treated 13 patients with congenital agranulocytosis with rhG-CSF initially using iv bolus or by continuous infusion. The patients are now being maintained on sc injections. In addition to sustained ANC of 1,000 cells/μL for more than 6 months, these patients have also experienced clinical resolution of preexisting chronic infections as well as a decrease in the number of new infections and, consequently, a decrease in the number of days they have required antibiotics.

Bone pain, once again, has been the major side effect observed in these patients. In addition, one patient complained of intermittent discomfort in chronically enlarged lymph nodes during the dose adjustment period when the white blood cell count was excessively high. Two of the original 5 congenital agranulocytosis patients (*11*) experienced an increase in spleen size.

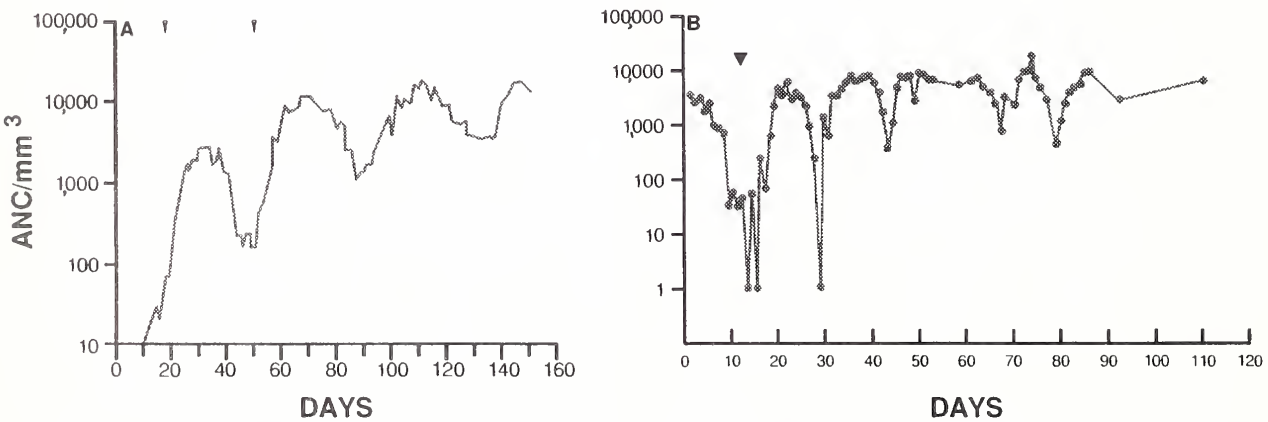

FIGURE 2.—Response in ANC of patient with idiopathic neutropenia (A) and in another with cyclic neutropenia who was treated with rhG-CSF. (B). A portion adapted with permission of the publisher (*10*).

Future studies may determine whether growth factors will have an impact on survival rates in malignant and nonmalignant conditions associated with myelosuppression. If one can maintain the dose and schedule of chemotherapeutic regimens, by reducing toxicity one may achieve improved antitumor response and have a positive impact on survival. In addition, by diminishing the side of effects of treatment, one can begin to test whether higher doses of chemotherapy can improve response rates and ultimately the survival of patients with cancer. Already, Bronchud et al. (*12*) have demonstrated that one can administer 75 μg doxorubicin/m^2 every 14 days to patients with breast and ovarian cancers, with enhanced recovery of myelosuppression when rhG-CSF is given concomitantly. Other studies designed to address these important questions are presently ongoing or are in the process of being designed.

When myelosuppression results in limiting chemotherapeutic dosage, use of these growth factors may alter the definition of maximum tolerated dose for new and previously tested drugs. The growth factors may also prove useful in augmenting the number of malignant cells recruited into S phase of the cell cycle, thereby enhancing the susceptibility of these cells to killing by cycle-specific agents such as cytarabine.

Clearly, CSF will be useful in cancer therapy as an adjunct to serotherapy with monoclonal antibodies, augmenting effector-cell function in the absence of tumors and improving host defense against infection. They may also help to prime donors for bone marrow transplantation; both G-CSF and GM-CSF have been shown to increase peripheral blood hematopoietic progenitors (*7, 11*). This should allow peripheral blood to be used as a vehicle for autologous or possible allogeneic transplantation, possibly obviating the need for bone marrow harvesting.

Finally, CSF and, in particular G-CSF and M-CSF, may be used to diminish leukemia cell self-renewal by promoting cell maturation. By promoting terminal cell maturation, one might be able to extinguish the leukemia clone.

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Brain, Behavior, and Immunity: An Interactive System

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ABSTRACT—The immune, neuroendocrine, and central nervous systems are stimulus response systems that are similar in the functions they subserve and tightly integrated in their actions. The reciprocal regulatory effects of these systems provide a basis (but not proof) for the belief that brain–immune interactions are of clinical relevance and not reducible to characteristics of component systems. In this paper, evidence for the integration and mutual regulation of central nervous and immune systems is reviewed. Additionally, behavioral and time-dependent neuroendocrine effects of interleukin-2 are presented in detail.—J Natl Cancer Inst Monogr 10:79–82, 1990.

No one would be surprised to hear that a specific form of immune system dysfunction called cancer can affect behavior. However, data that have emerged over the last decade suggest that the relationship between the immune and central nervous systems is far more intimate and their actions far more interdependent than could ever be suspected on the basis of observations of "illness behavior." Indeed, the immune, central nervous, and neuroendocrine systems are amazingly similar in the functions that they subserve and appear tightly integrated in their actions. [It has been suggested that the integration of these systems justifies their consideration as a single system (*1*); however, for clarity, I will continue to refer to them as separate systems.]

The immune system functions as a sensory and effector organ (*2*) as do the other two systems. It is a sensory organ to the extent that it recognizes foreign antigens, just as the endocrine and nervous systems recognize incoming signals. It functions as a stimulus response system, displaying remarkable specificity; antigens are "remembered," resulting in an altered (frequently enhanced) response upon reexposure to the antigen. The immune system processes the antigenic signal to regulate the nature and extent of the immune response. It also transmits signals and information (communicates) about the antigen and the resultant immune response to other elements of the immune system as well as to the central nervous and neuroendocrine systems. Conversely, the immune system can act as the recipient of afferent signals from the nervous and neuroendocrine systems that can influence and direct the nature or extent of the immune response. The high degree of similarity and integration of the immune, central nervous, and neuroendocrine systems is teleologically sensible, because their common task is to preserve homeostasis and assure the constancy and integrity of body cells and tissues, a task for which integration and regulatory redundancy are obligatory.

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IMMUNOMODULATION OF CENTRAL NERVOUS SYSTEM AND NEUROENDOCRINE ACTIVITY

The ability of immune events to modify brain and neuroendocrine activity has been inferred from two general types of observations. First, alterations in brain electrical activity (preoptic/anterior hypothalamic area, periventricular nucleus) and regional neurotransmitter (norepinephrine) concentrations have been observed to accompany immune activation (*3–5*). Second, cells of the immune system have been observed to synthesize and secrete classical hormones as well as immunohormones (lymphokines and cytokines) that appear capable of altering hypothalamic and pituitary function (*6*). Thus, in addition to its role as stimulator of lymphocyte interleukin-2 secretion, interleukin-1 stimulates corticotropin and cortisol secretion following peripheral administration (*7, 8*), induces slow-wave sleep (*9*), and, as an "endogenous pyrogen" (*10*), produces fever by stimulating hypothalamic thermoregulatory centers. Interferon-alfa has been observed to produce a number of central effects including the following: catalepsy, decreased locomotor activity, and analgesia (*11*); increased synchronization of electroencephalogram and decreased electrical activity in the preoptic/anterior hypothalamic area following intracerebroventricular injection (*5*); modulation of opiate tolerance and withdrawal (*12*); an opioid-mediated increase in neuronal discharge in the hippocampus and medial basal hypothalamus following iontophoresis (*13–15*); and production of neuropsychiatric symptoms following iv administration in humans (*16*). Other cytokines such as glucocorticoid inhibitory factor have in vitro effects on endocrine function, the clinical relevance of which is undetermined. A clearly defined role in either immune or central nervous system processes is similarly absent for the impressive list of neuropeptides produced by the immune system. Nonetheless, it has been suggested that these immune soluble products mediate the effect of the immune response on brain regions that, in turn, modulate the secretion of neuroendocrine factors that can influence the immune response (*5*). Additionally, the potential significance of these immune endocrine secretions is suggested by the report of inflammation-induced Cushing's syndrome in hypophysectomized mice (*17*).

NEUROENDOCRINE MODULATION OF THE IMMUNE RESPONSE

The ability of neuropeptides to alter immune function has been widely described (*18*) and includes modulation of lymphokine production (corticotropin), lymphocyte migration (bombesin), stimulation of natural killer cell activity (beta-endorphin), modulation of phagocytosis and lymphocyte proliferation (substance P), suppression of T-cell function and reduc-

tion of cell-mediated immunity (estrogen), and inhibition of lymphocyte proliferation (somatostatin) and lymphoblastic transformation (vasoactive intestinal peptide). Furthermore, Smith et al. (*19*) reported that several endorphin and enkephalin analogues had differential effects on antibody synthesis, mitogenesis, and natural killer cell activity. The physiologic significance of these neuropeptide–immune effector cell interactions is suggested by reports on lymphocytes of specific neuropeptide binding sites, e.g., beta-endorphin, somatostatin, substance P, estrogen, vasoactive intestinal peptide (*18*).

IMMUNE MODULATION BY THE CENTRAL NERVOUS SYSTEM

The effects of central nervous system activity on immune events can, for convenience, be subdivided into three areas. First, immune alterations have been reported in response to stimulation or electrolytic lesions of specific brain regions. Thus Roszman et al. (*2*) produced alterations in various immune parameters, including spleen cell number, thymocyte number, natural killer cell activity, and antigen responsiveness, alterations that occurred after a brain lesion in a region-specific fashion following lesions of the anterior hypothalamus, hippocampus, or amygdala. Hypophysectomy was observed to block the inhibitory effects of anterior hypothalamic lesions on splenic cell number and reactivity, but it did not alter the induced suppression of thymocyte mitogen activity. Thus at least some brain-related neuroimmunomodulation does not require mediation through pituitary peptides. Second, alterations in a number of in vitro immune measures have been observed following application of a variety of stressors. The type and direction of immune alteration observed are highly dependent on the nature, intensity, and pattern (e.g., acute vs. repeated exposure) of the stressor as well as on the sex of the recipient of the stress, particularly in animals (*20*). Reduced natural killer cell activity, total lymphocytes, T cells, and helper T cells, as well as increased neutrophils have been reported in different studies of stress and depression. However, a troubling absence of consistency characterizes studies of immune function in stress and depression. Although discrepant findings may reflect the failure to control for as yet undiscovered modulators of immune activity, a still more disturbing problem is our ignorance of the clinical correlates or significance of relatively subtle alterations in in vitro immune measures. Thus not only is it unclear whether the immune "disturbances" described in studies of stress and depression are detrimental or beneficial, it is unclear if they are in any way clinically meaningful or, indeed, reflective of the in vivo state. Given the intimate and complex interactions that exist among the immune, central nervous, and neuroendocrine systems, the immunologist's test tube hardly recreates the environment in which immune regulation is accomplished (Ader R: Personal communication).

A particularly fascinating set of observations emerged from a series of studies of the immune response to uncontrollable aversive stimuli. In these studies, animals were subjected to repeated aversive stimuli (e.g., tail shock or foot shock) and were "yoked" to animals receiving the identical shock but who, by learning the proper response (e.g., lever pressing), could terminate the shock delivered to both animals. The animals unable to control the shock were reported to develop a number of biologic changes not seen in those that received identical shocks at the identical time but were able to control the aversive stimuli. These biologic changes included depletion of forebrain norepinephrine (*21*); immune incompetence, as assessed by the inability to reject implanted tumors (*22*); decreased lymphocyte thymidine uptake (*23*); and development of opiate-mediated, poststress analgesia (*24*). Obviously, these findings were exciting because they implied that it was the perception of and response to stress (rather than the characteristic of the stressor) that determined the physiologic consequence of repeated exposure to stressors. However, Maier and Laudenslager (*25*) have recently raised serious concerns about this phenomenon. These concerns include the following: 1) The reduced proliferative response to mitogen stimulation following inescapable shock is not reliably replicated. 2) Because researchers frequently use only a single stressor and a single measure of immune function and, further, only rarely provide internal replication, one is unable to determine whether an isolated finding represents an accidental combination of favorable conditions or a finding that is easily replicable. 3) The proliferative response to mitogen stimulation is extremely variable and overwhelms even large effects of stressor exposure. 4) The conditions and duration of in vitro measures minimize the impact of those neural and endocrine factors that are most likely to mediate an impact of a stressor on immune activity. 5) Given the dynamics of lymphocyte pooling and migration, the collection of cells from a single site represents a significant sampling bias that may enhance cross-study discrepancies. The learned helplessness model continues to offer promise as a means for exploration of the bidirectional nature of the relationship between biology and perception. Nonetheless, as suggested by Maier and Laudenslager, enthusiasm must be tempered by caution until existing methodologic concerns have been addressed.

A third area of investigation has been derived from Ader and Cohen's (*26*) initial observations of the conditioning of cell-mediated immunity. These investigators conditioned taste aversion by pairing saccharin (conditioned stimulus) with cyclophosphamide, an unconditioned stimulus that caused gastrointestinal distress. The animals learned to avoid drinking a substance (saccharin) that they ordinarily found desirable because of the gastrointestinal discomfort that they came to associate with it. In a subsequent study, the investigators learned to their surprise that the animals that they were preparing for the study (with structured light–dark cycles and saccharin ad libitum) mysteriously died. Ader and Cohen discovered that the animals that died were those that had previously taken part in the conditioned taste aversion study. Whereas the animals had long since "forgotten" their taste aversion response to saccharin, their immune system appeared to "remember" the association between saccharin and immunosuppression. Thus a cyclophosphamide-induced immunosuppression became conditioned to what initially was an innocuous stimulus, saccharin. This provocative observation of conditioned immunosuppression has subsequently been replicated by Ader and Cohen as well as other investigators (*27, 28*). The potential therapeutic relevance of these findings is in part suggested by the demonstration (*27*) of a reduced need for immunosuppressive medication in mice with an otherwise fatal autoimmune disease when the animals were conditioned to suppress their immune response after ingestion of saccharin. Animals that received equal amounts of cyclophosphamide and saccharin (but their cyclophosphamide and saccharin doses were not paired to produce a

conditioned response) were less immunosuppressed than the saccharin-conditioned, immunosuppressed animals and died of their autoimmune disease significantly more frequently. This well-controlled, well-designed study suggests that conditioning (intentional or, potentially, fortuitous) alters immune function to an extent that is clinically significant.

Although studies of learned helplessness and conditioned immunosuppression suggest the ability of the central nervous system to modulate the immune response, results of research on lymphokine administration in human immunotherapy protocols have revealed substantial effects of immune soluble products on central nervous system function and behavior. Various reports have appeared that describe neuropsychiatric dysfunction, including cognitive impairment, mania, delirium, and catatonia (*29–31*), in association with interferon-alfa administration. One recent report (*29*) suggests that interferon-alfa-induced neurotoxicity is most likely to occur in the setting of preexisting neurologic dysfunction. As we had observed treatment-limiting behavioral and cognitive abnormalities following immunotherapy with interleukin-2 and lymphokine-activated killer cells, we systematically investigated (*32*) the neuropsychiatric side effects that accompanied administration of interleukin-2 in 44 patients who participated in an immunotherapy protocol. Patients were rated just prior to and at the conclusion of each 5-day treatment phase. Twenty-two of 44 patients showed severe cognitive changes in the form of disorientation and dramatic deterioration in cognitive performance compared with their base-line status. All these patients met criteria for delirium, as described in the *Diagnostic and Statistical Manual* (3rd ed) of the American Psychiatric Association. Fifteen patients showed severe behavioral changes lasting from 24 hours to several days and required the use of neuroleptic or physical restraint. Seven patients developed frank delusions during treatment. Overall, 66% of the patients developed moderate to severe cognitive or behavioral problems during interleukin-2 treatment, with these neuropsychiatric problems developing almost exclusively at the end of the 5-day treatment phase. Symptoms usually resolved within several days after treatment was stopped. The neuropsychiatric side effects appeared clearly to be dose dependent; those patients receiving 30,000 U/kg were more likely to experience only mild cognitive effects or no neuropsychiatric changes compared with those receiving the high-dose treatment (100,000 U/kg). None of the factors examined, including interleukin-2-associated weight gain, past psychiatric history, and interleukin-2-induced renal or hepatic dysfunction, were found to be predictive of the development of neuropsychiatric toxic reactions. Thus it appeared that interleukin-2 could directly affect central nervous system function or, given the latency of the development of neuropsychiatric changes, could indirectly affect brain activity. This might occur by enhancing the accumulation of behaviorally active substances (metabolic effects of interleukin-2) or by increasing the permeability of the blood–brain barrier, thereby increasing the exposure of the central nervous system to behavior-altering factors. As we were unable to demonstrate an interleukin-2-induced alteration in the permeability of the blood–brain barrier in rats to either large-molecular-weight (albumin) or small-molecular-weight (alpha-aminoisobutyric acid) markers, we undertook a second study to examine the endocrine effects of interleukin-2 administration.

In a group of 30 patients receiving either interleukin-2 only or followed by lymphokine-activated killer cells, blood samples were obtained immediately before and 4 and 8 hours following its administration on the first and fourth days of treatment. Blood samples were assayed for the stress axis hormones corticotropin-releasing factor, corticotropin, beta-endorphin, and cortisol. It was hypothesized that basal or stimulated hormonal changes are associated with or predict the interleukin-2-related neuropsychiatric changes. Clear-cut stimulated elevations of corticotropin, beta-endorphin, and cortisol were observed 4 hours after interleukin-2 administration on the first day of treatment, with less but significant elevation also observed on the fourth day of treatment (*33*). What was most striking, however, was the order of magnitude greater stimulated increase in corticotropin and beta-endorphin on day 1 of interleukin-2 administration in patients who had also received it in an earlier treatment course. The mean increase in stimulated hormone levels, from baseline to 4 hours after interleukin-2 infusion, for patients in the repeat treatment course versus the first treatment course was roughly tenfold for beta-endorphin and twentyfold for corticotropin. Although repeated administration over the course of 4 days resulted in a slight attenuation of the interleukin-2-stimulated hormonal response, re-exposure to it following a treatment-free interval (6 days to 3 mo) resulted in profound elevations above baseline for corticotropin (mean change = 600 pg/mL) and beta-endorphin (mean change = 100 fmol/mL). The marked course-related increases in these hormones could suggest an anamnestic response to interleukin-2 by the immune system or, alternatively, at the hypothalamic–pituitary–adrenal axis. This phenomenon parallels the neurochemical changes that have been described by Antelman (*34*) as reflecting time-dependent sensitization. According to Antelman, the effects of a medication may represent the "culmination of an exclusively time-dependent process" initiated by acute exposure to a drug, with re-exposure after an extended interval resulting in a significantly greater effect than that first observed. Preliminary analysis has revealed no clear relationship between the degree of hormonal stimulation and the severity of neuropsychiatric changes experienced. However, lymphokine-associated hormonal sensitization may, through increased levels of stress hormones, modulate the antitumor effects of interleukin-2 and potentially explain some of the variance in clinical response to immunotherapy. At the very least, these data provide further evidence of a link between the neuroendocrine and immune systems and suggest that the immune system "sensitizes" or "conditions" the neuroendocrine system. An immune system event, then, may not only influence neuroendocrine regulation acutely, but may also dramatically alter subsequent neuroendocrine response for a long time thereafter (in this case, up to 3 mo later).

CONCLUSION

Significant evidence has been accumulated from animal and in vitro studies to suggest that the immune, neuroendocrine, and central nervous systems are integrated and interdependent components of homeostasis. However, the clinical significance of these intersystem interactions is far from clear. We are not in a position to know whether our psychologic response to stressors or illness may significantly influence immune surveillance or activity, although evidence (both empirical and experimental) suggests that we think and feel differently as a product of our immune response.

Despite considerable methodologic difficulties that continue to limit the generalizability of reported psychoneuroimmune interactions, it seems clear that the immune, neuroendocrine, and central nervous systems do not function in isolation and that major perturbations of any single system will be registered by and influence the activity of the remaining systems. Emergent properties of higher order systems are not reducible to the characteristics of their components. With continued advances in our understanding of both component and emergent processes, our as yet unrealized wish to understand and exploit the relationship between health and behavior will approach attainment.

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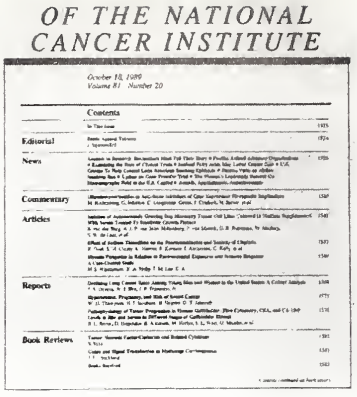
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NCI Monographs No. 8

Smokeless Tobacco Use in the United States

In its first comprehensive review of the relationship between smokeless tobacco and health, the Public Health Service identified three major health risks associated with the use of smokeless tobacco: oral cancer, development of leukoplakias and other oral lesions, and nicotine addiction.

The Surgeon General has stated that it is imperative that smokeless tobacco not be allowed to add to the unnecessary toll of over 350,000 lives lost prematurely each year due to cigarette smoking. The efforts professionals and private organizations launched to decrease the number of cigarette smokers must be expanded to include users of smokeless tobacco, especially those users who are under 21 years of age.

As part of the National Cancer Institute's strategy to reach the goal of reducing cancer morbidity and mortality by 50% by the year 2000, the Smoking, Tobacco, and Cancer Program of the Division of Cancer Prevention and Control funds research for the development and evaluation of intervention programs. The papers in this monograph present the collaborative efforts of researchers in their quest for effective preventive measures, reviews of cancer risk associated with smokeless tobacco, reports of current use by adolescents and adults, reports of promotion activities by producers, and a discussion of future directions for research.

This publication is available **only** from the U.S. Government Printing Office. Please include the Stock No. 017-042-00255-9 and your check or money order for $5.50, made payable to the Superintendent of Documents, and mail your order to the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402-9325.

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